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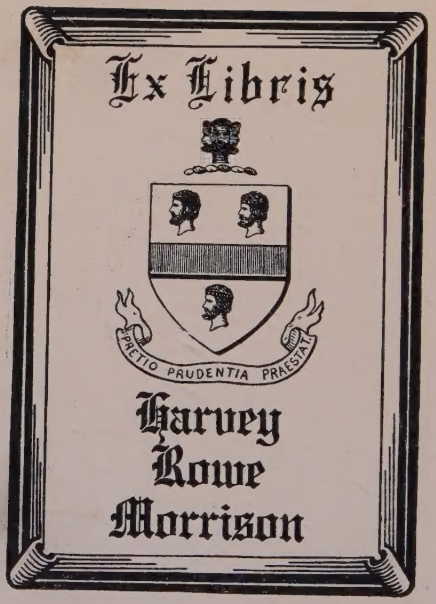
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RECENT ADVANCES IN PHYSIOLOGY

BY

C. LOVATT EVANS

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Jodrell Professor of Physiology and Fellow of University College, London

FOURTH EDITION

WITH 113 ILLUSTRATIONS

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PREFACE TO THE FOURTH EDITION

In preparing this edition, two new chapters have again been added, and two of the former ones removed; nine of the chapters of the first edition are still retained, though with considerable revision.

I wish to thank numerous friends and colleagues who have made suggestions for improvements, and especially Dr. P. Eggleton for help with pp. 148–158, Dr. W. Rushton for advice in chapter X, Dr. L. Gross for Figs. 1 and 2, Professor C. Heymans for Figs. 13–21, Messrs. C. F. Palmer for Figs. 3 and 50, and Mr. H. P. Gilding, M.A., B.M., for reading the paged proofs.

Again I would thank Messrs. J. & A. Churchill for their unfailing help and the printers for the excellence and accuracy of their work.

C. LOVATT EVANS.

University College, London.

PREFACE

THIS book may be called an Elementary Text-Book of Advanced Physiology. Its aim is to present to the student who has worked through an ordinary text-book an account of some of the problems with which physiologists have been concerned during recent years, and thus to serve, not only to enrich the student's knowledge as regards subjects of contemporary interest, but also to form a convenient bridge by which he may, if he feels so disposed, pass more easily into the original literature of those subjects.

I trust, therefore, that it may prove useful, not only to the more seriously minded students reading for an intermediate examination for a medical degree, but also may serve as a preliminary work for the guidance of students preparing for honours examinations.

If any of my physiological friends read, and admit having read, this book, I imagine they will tell me that I have not dealt in sufficient detail with the branches of the subject in which they themselves have specialised, or that the version of those branches which I have presented is a biassed one. I must plead as a sufficient excuse the magnitude of my own ignorance, and, reminding them that it is human to err, ask them to forgive me. For I have not presumed to instruct them, but only students. But if any of my colleagues do find the book of interest or value, I shall be immensely flattered by their good opinion.

In preparing this work, I have found the articles in *Physiological Reviews* of the greatest help, and hereby record my indebtedness to all those authors whose writings I have taken the liberty to utilise.

I cannot let pass this opportunity of recording my grateful thanks to Dr. G. V. Anrep, the distinguished pupil of Professor Pavlov, for the help which he has so generously given me in the preparation of the chapter on the conditioned reflexes, which is almost entirely the result of his direct help, and of the talks I have had with him on the subject.

Thanks are also due to Professor J. N. Langley, F.R.S., Editor of the *Journal of Physiology*, and to the various authors concerned, for permission to publish many figures, duly recognised in the text.

Also to Professor R. Magnus, of Utrecht, for his kindness in allowing me to reproduce Figs. 54 to 64 from his monumental work, "Körperstellung," and similarly to the publishers, Messrs. Springer, of Berlin, for those figures, and for Fig. 29, taken from Krogh's work on capillaries; Professor W. B. Cannon, of Harvard, and Messrs. Appleton, of New York, for permission to reproduce Fig. 36; Professor E. Weiss and Messrs. Urban and Schwazenberg for Fig. 27; Drs. G. V. Anrep and D. T. Harris for Figs. 1, 11, and 47 from their "Practical Physiology"; the editors and publishers of the *British Journal of Experimental Pathology* and the *Biochemical Journal;* Drs. Gye and Purdy, Dr. Dale, Sir F. Gowland Hopkins, and other authors who have so kindly allowed me to use their figures from those publications.

C. LOVATT EVANS.

St. Bartholomew's Medical
College, London.

August, 1925.

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USEFUL NUMERICAL CONSTANTS

Prefixes used in nomenclature, in addition to usual ones :—

Micro $= 10^{-6}$ *i.e.*, one-millionth.
Milli $= 10^{-3}$ *i.e.*, one-thousandth, etc.
Kilo $= 10^{3}$ *i.e.*, one thousand.
Mega $= 10^{6}$ *i.e.*, one million, etc.
E.g., 1 Kilo Calorie $= 1$ Calorie $= 10^{3}$ calories.
1 Micro calorie $= 10^{-6}$ calories.

---

1 γ = 1 microgramme $= 10^{-6}$ grams, *i.e.* $\frac{1}{1000}$ mg.
1 σ $= 0{\cdot}001$ second.
1 farad = an electrical capacity which holds 1 coulomb when charged to 1 volt.
1 microfarad (μF) $= 10^{-6}$ F.
1 μ $= 10^{-3}$ mm. (= 1 micrometre).
1 $\mu\mu$ $= 10^{-6}$ mm. (= 1 millimicrometre).
1 Ångström unit $= 10^{-7}$ mm.
1 cal. { = heat to raise 1 gram of water through 1° C.
{ $= 4{\cdot}2 \times 10^{7}$ ergs $= 42{\cdot}6 \times 10^{3}$ gram cms.
1 micro cal. $= 42$ ergs $= 42{\cdot}6 \times 10^{-3}$ gram cms.

Kinetic C.G.S. unit of force = 1 dyne = force which, acting on mass of 1 gram, causes it to move with an acceleration of 1 cm. per second, per second.

Gravitational unit of force = 1 gram weight = 981·5 dynes.

Kinetic unit of work = 1 erg = 1 dyne-cm. $= 23{\cdot}84 \times 10^{-9}$ calories $= 10{\cdot}16 \times 10^{-4}$ gram cms.

Gravitational unit of work = 1 gram cm. $= 981{\cdot}5$ ergs $= 23{\cdot}4 \times 10^{-6}$ gram cal. $= 23{\cdot}4$ micro cals.

Kinetic Unit of Power = 1 erg. per second.
1 kilowatt $= 10^{3}$ watts $= 10^{10}$ ergs per second.

Gravitational Unit of Power = 1 gram-cm. per second.
1 horse-power $= 0{\cdot}746$ kw $= 457$ kg-m. per minute.
1 mole = 1 gram molecule $= 6 \times 10^{23}$ molecules.
Molar = 1 gram molecule per litre.
Millimolar $= 10^{-3}$ gram molecules per litre.

Osmotic pressure of molar solution (undissociated) $= 22{\cdot}4$ atmospheres.

Osmotic pressure of binary electrolyte (whose degree of dissociation $= \delta$) in molar solution $= (1 + \delta) \times 22{\cdot}4$ atm. $= (1 + \delta) \times 22{\cdot}4 \times 760$ mm. Hg.

INTRODUCTION

THE ADVANCE OF PHYSIOLOGY

Physiological knowledge falls under two headings, which may be called descriptive and theoretical. The former consists of statements of facts, and much of the older physiology is of that type. If we say that the blood circulates, or that the heart beats, or that blood clots, we are stating facts. Entering into a more detailed description, we might say that blood circulates along such and such a route, or at such and such a rate, that certain conditions hasten the rate of the heart beat or retard it, that blood clotting is accelerated or retarded by particular procedures. All this, provided the observations have been accurately made, is incontrovertible, because it is mere description.

Physiology as a science, having accumulated a large number of facts, seeks to inter-relate them, and looks for the explanation of those facts. As soon as this attempt is made, we pass from the descriptive to the theoretical aspect of physiology. When we say that the blood circulates because the heart beats, we have made a statement which admits of either proof, qualification, or refutation, according to the results of experiments planned to test the hypothesis. The answer to one question naturally raises another, and it is obvious that there can be no final limit placed to our curiosity.

Since there can be no ultimate solution and explanation for all things, physiology would certainly consider itself well satisfied if it could explain its phenomena in terms such as those conventionally used in the explanation of the natural phenomena of the non-living world, that is to say, in terms of physics and chemistry. If we could accomplish so much,

our goal could, indeed, be considered to have been reached, and the responsibility for final explanations could no doubt be passed from chemistry through physics to the realms of an enlightened philosophy.

We can rest quite securely in the belief that even this provisional goal will never be reached; closer and closer inspection of any natural phenomena, particularly those of living things, always reveals greater and greater complexity and finer and more unexpected inter-connections of phenomena. The pretty conceits of a century ago appear as absurd to us as our fancied omniscience will seem to our successors a century hence. The limit to the advance of knowledge will be set, not by the unwillingness of Nature to reveal, but by the inability of the mind to grasp her complexities.

It is because physiology must seek its theoretical explanations in terms of physics and chemistry that the most satisfactory advances which have been made, or which are likely to be made, in theoretical physiology are along lines of enquiry which utilise the recognised methods of those sciences. A rich harvest has already been reaped by the application of chemical methods, and it is likely that the similar use of physics will, in the lifetime of the present generation of physiologists, also give much new theoretical matter. Already the subject of physiology has reached such a degree of complexity that it threatens to break up into a group of specialist subordinate branches. While this disintegrative tendency is greatly to be deplored, it is in the end inevitable, if progress is to continue. Already several thousand papers dealing with physiology or related subjects appear annually, and, even granting that the majority of them may have no more than local or temporary interest, their diluent effect is enormous.

The advance of theoretical physiology is intimately related to the advance of medicine, for it is the only basis on which scientific as distinct from empirical medicine can

possibly rest. There can be no great theoretical conceptions in medicine without corresponding great underlying physiological principles. The conquest of physiology by chemistry and physics is the aim of the physiologist, and its advent depends on the success of his efforts to apply to his own subject the findings of chemistry and physics. Whether, or rather when, the conquest of medicine by physiology eventuates, will depend as much on the perspicuity of pathologists and physicians in applying physiological theory as on the diligence and intelligence of physiologists in developing it.

Physiology is often considered to be too highly academic a subject, and this accusation is not infrequently levelled at it by self-styled men of practical common sense. So was electricity regarded by the empiricists of the period which saw its early development.

As it is no direct affair of the physiologist to find and work out the innumerable applications of his subject to medicine, surgery, sociology, anthropology, biology, and many other subjects which its advances touch more or less intimately, we must be content in a volume of the scope of the present book to deal with physiology as a science and only to make references to its applications to other branches of knowledge in so far as these applications reflect back light to physiology itself; which often happens.

RECENT ADVANCES IN PHYSIOLOGY

CHAPTER I

THE CORONARY CIRCULATION AND ITS REGULATION

The plentiful supply of blood demanded by the heart muscle is delivered to it by the two coronary arteries. These are relatively large vessels which branch freely and show frequent anastomoses between their branches. Some idea of the richness of the coronary circulation can be seen from Fig. 1, which is an X-ray photograph of a heart the arteries of which have been filled with opaque material.

The left coronary artery has two main branches, the circumflex, which runs to the left, and the descendens, which passes in the anterior interventricular sulcus to the apex. The right coronary artery runs to the right in the auriculo-ventricular sulcus, and ends in two smaller branches. Many of the terminal branches of the right and left coronary arteries anastomose freely with one another. The larger branches are accompanied by veins, and by nerves from the cardiac plexus.

Blood is returned from the heart chiefly into its own right auricle by venous channels, of which the largest is the coronary sinus (Fig. 2). All the other veins, *e.g.*, posterior cardiac vein, veins of Thebesius, etc., draining definite areas also open into the heart cavities, the right auricle, and the right, or even left, ventricle.

An investigation of the Thebesian veins by Grant and Viko showed that the orifices of these vessels may be found in any

part of the endocardium, but specially on the interauricular and interventricular septum, mainly on the right side. The Thebesian veins, as shown by these researches, exhibit three main types of branching. In the first, or " Thebesian tree " type, the smaller tributaries form an independent branching system in the endocardium and underlying muscle ; in the second type there are Thebesian intercommunicating vessels, by which one or more of

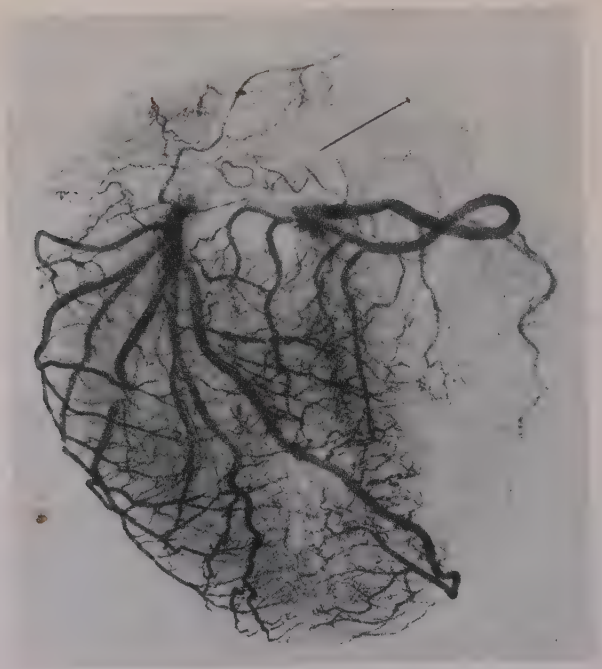

FIG. 1.—X-ray photograph showing the arterial supply to the heart, viewed from behind. (L. Gross.)

such veins anastomose ; while in the third group there are Thebesian-coronary communicating vessels by which the Thebesian veins communicate with branches of the coronary veins. Communication between the branches of the coronary arteries and the Thebesian veins are never direct, but only by way of the capillaries and coronary veins. As an anomaly, however, cases have been described in which branches of the coronary artery communicate with a ventricle.

The fact that there are veins opening into the ventricles, and especially into the left ventricle, strikes one at first as somewhat of an oddity, until one recollects that, whereas the pressure in the coronary system has always a positive value, that in the ventricles is practically at zero for a good part of

FIG. 2.—X-ray photograph to show the venous drainage of the heart, viewed from behind. (L. Gross.)

the cardiac cycle; that those veins can function intermittently is therefore beyond question, and, as we shall see later, the same is no less true of all the other venous channels in the heart.

There are many venous as well as arterial anastomoses in the heart, and, as Grant and Jones have shown, even the

coronary sinus may be blocked without such interference with the coronary circulation as to occasion death.

Methods of Investigation.—The investigation of the conditions which control the coronary circulation has been greatly facilitated by the use of two experimental methods, namely, the heart-lung circulation of Starling and the coronary cannula devised by Morawitz and Zahn. A sketch of the cannula is shown in Fig. 3.

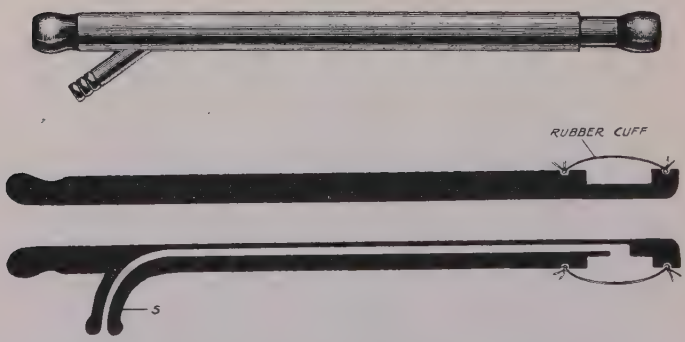

FIG. 3.—Upper figure: the Morawitz coronary cannula (C. F. Palmer, Ltd.). Lower figure: Diagram of the Morawitz coronary cannula.

This cannula is readily introduced through an opening made into the right auricular appendix, and can be inserted into the coronary sinus without appreciable loss of blood. It is fixed in position there by the inflation to a suitable extent of the small rubber cuff placed near to its end. This inflation is effected by injecting saline solution or water into the small side tubes by means of a syringe. Once this cannula is inserted, the whole of the coronary sinus blood flows away through the cannula and can be collected. The blood should, of course, be defibrinated, or rendered incoagulable by the use of hirudin or heparin.

This cannula has been used by several workers for the study of the coronary circulation in the intact animal, or in

the heart-lung preparation, and much that was previously obscure has been illuminated by this technique.

The Fraction of Drainage by the Coronary Sinus.—It was assumed by Morawitz and Zahn that the whole of the cardiac venous return was collected by their sinus cannula, but it was shown by Evans and Starling that the coronary sinus only drains away approximately three-fifths of the whole

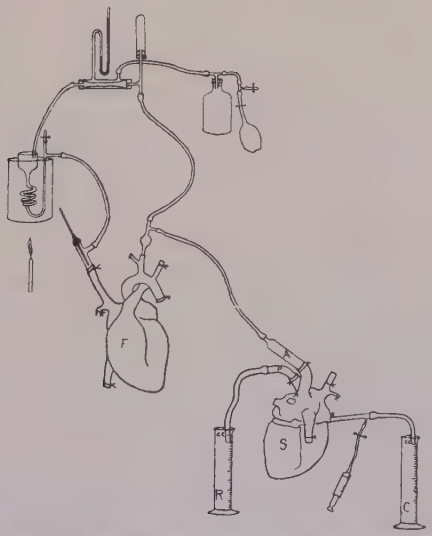

FIG. 4.—Determination of the fractional flow through coronary sinus. (Evans and Starling.)

coronary flow; the fraction drained is independent of the total magnitude of that flow. This determination was carried out in the following manner (Fig. 4).

The heart-lung preparation (F) was made from a large dog, and from the arterial side of this the heart (S) of a small dog was perfused from its aorta, the cannula pointing backwards towards the heart, as in Langendorff's method of perfusion, so that the arterial valves were closed and the blood was delivered only into the coronary arteries. After

passing through the coronary vessels of the second heart, this blood is delivered almost entirely into its right ventricle, so that if we ligature all the other vessels on the right side of the subject-heart and introduce a cannula (P) into the pulmonary artery, the coronary blood will issue from this cannula and its rate of flow can be measured. This gives the total coronary flow (apart from the small fraction which enters the left ventricle). The Morawitz cannula is then introduced into the coronary sinus in the usual way, and the flow through it also collected and measured; the blood which now issues from the pulmonary artery represents that which has been drained away by the veins other than those opening into the coronary sinus: this may be called the "remainder flow." It was found in this way that about three-fifths of the total flow is returned by the coronary sinus, and two-fifths by the various other venous channels.

Under the influence of adrenalin or asphyxia, both of which agencies widely dilate the coronary vessels, and so give a great increase in the total flow of blood through the heart, the ratio of coronary flow to remainder flow remains about the same. This is shown in the following table :—

Distribution of Coronary Flow. (Evans and Starling.)

Flow in cubic centimetres per minute; S from coronary sinus, R from remainder. S + R = total flow. Irrigation coefficient = c.c. of total blood flow per 100 gm. of heart.

Subject heart, 48 gm. Arterial blood-pressure of feeder, 80 mm. Hg.

Time.	Pulse rate.	*S*	*R*	*S* + *R*	*S*/*R* + *S*	Irrigation coefficient. per cent.
2·55	—	21·4	11·9	33·3	0·64	69
2·58	—	20·7	11·9	32·6	0·63	68
3·04	—	23·5	11·9	35·4	0·66	73
3·06	133	21·3	—	—	—	—
3·10	1 c.c. adrenalin 1 : 10000 added to blood of feeder.					

Time.	Pulse rate.	*S*	*R*	*S* + *R*	*S*/*R* + *S*	Irrigation coefficient. per cent.
3·12	213	34·5	21·6	56·1	0·62	117
3·14	196	45·5	23·2	68·7	0·66	140
3·17	187	36·6	23·3	59·9	0·61	123
3·18	Artificial respiration of feeder stopped, to induce asphyxia.					
3·20	162	34·5	21·5	56·0	0·62	117
3·22	150	37·0	24·3	61·3	0·60	127
3·24	154	43·7	28·3	72·0	0·61	150
3·27	158	89·5	60·0	149·5	0·60	302
3·28	150	111·0	66·5	177·5	0·63	370

It is seen that in this experiment the fraction drained by the coronary sinus (S/R + S) varies from 0·6 to 0·66.

In these experiments, it is true that the subject-heart was not pumping blood as it normally would be, but recent observations by Anrep and others indicate that much the same distribution of blood obtains when the heart is beating under more normal conditions. In subsequent experiments involving the use of the Morawitz cannula, it has therefore been generally assumed that the total coronary flow is five-third times the flow through the coronary sinus cannula.

In the recent experiments of Anrep, Blalock and Hammouda, the coronary arteries were perfused with blood at any desired pressures by directly tying cannulæ, one into the right artery and one each into the circumflex and descendens branches of the left. The inflow into these was measured and compared with the outflow from the coronary sinus, the heart being allowed to pump blood from both ventricles in the normal manner, with any desired output or aortic pressure. They found that the coronary sinus fraction of the total flow was of the same order as that found by Evans and Starling, and was only slightly affected by changes of the aortic pressure, coronary pressure, rate or output of the heart, or of temperature, and was also much the same in the fibrillating or standstill heart as during normal beating.

When there was considerable rise of the intraventricular pressure so as to cause overfilling of the ventricles, however, the coronary fraction may fall to about 50 per cent., because then less blood flows *viâ* the coronary sinus, and more by other venous channels.

The relative distribution of blood between the different coronary arteries and the amount of that from each artery which enters the coronary sinus were also found to be approximately constant, thus, only about 40 per cent. of the flow through the right coronary artery reaches the sinus, while about 70 per cent. of that by the left artery reaches it. About 80 per cent. of the whole flow to the dog's heart was found to be carried to it by the left coronary artery. When one artery, or a main branch, such as the circumflex, was obstructed, they obtained no evidence of increased flow along other large arteries, as might have been expected if immediate anastomotic connections were established with them : such anastomoses as exist are apparently not competent to enable the heart to receive a maximal supply of blood when a large branch is occluded : this is, indeed, what might be expected, and does not mean that the anastomoses may not be able, by directing part of the supply from other parts, to tide an area of the myocardium over a period of temporary obstruction.

The Coronary Circulation during various Phases of the Cardiac Cycle

The hæmodynamics of the circulation of blood through the coronary vessels is complicated by the occurrence of the rhythmic contractions of the myocardium, by the great and rapid changes of pressure which occur in the chambers of the heart, and possibly by the disposal of the aortic valves near to the orifices of the coronary arteries. It is therefore only by the application of exact methods of investigation that we can decide whether, or to what extent, the events of the cardiac cycle subject the circulation in the heart itself to

conditions quite different from those in other parts of the body; whether, for instance, the flow into the coronary arteries, say during systole, is greatest because the arterial pressure is then at its height, or least owing to the compression of the vessels by the contracting heart muscle. Similar considerations may be applied to the conditions of outflow from the sinus and other veins. Of the many investigators who have studied this aspect of the coronary circulation, the majority perhaps held the opinion, supported by Porter and by Langendorff, that, though there was a higher pressure in the coronary arteries during the ejection phase of systole, the ventricular contraction soon reduced the rate of inflow, but increased the rate of outflow, by squeezing blood out from the venous side; during diastole, the outflow was thought, at all events at first, to be checked, since the emptied vessels had to be refilled, the inflow meanwhile increasing. The arrangements in these earlier experiments, however, were really not adequate for the solution of the problem, and though the recent work of Anrep and his collaborators has on the whole confirmed this previous work, it has also added to it in many particulars and modified it in others.

Methods of Investigation of Inflow and Outflow

The methods employed by Anrep and his collaborators enabled the rate of outflow from and of inflow into the coronary vessels of the heart-lung preparation to be recorded either simultaneously or separately. For this purpose hot wire anemometers were employed. The principle of this instrument is that the electrical resistance of a wire depends on its temperature; if a fine wire is electrically heated and placed in one arm of a Wheatstone bridge, it may be used to detect, and record by a string galvanometer, the passage of a current of air over it, since this will cool it at a rate depending on, and roughly proportional to, the velocity with which the

air moves. All that is needed, therefore, is to make the flowing blood displace air over the hot wire and to record the resulting variations of resistance by a string galvanometer.

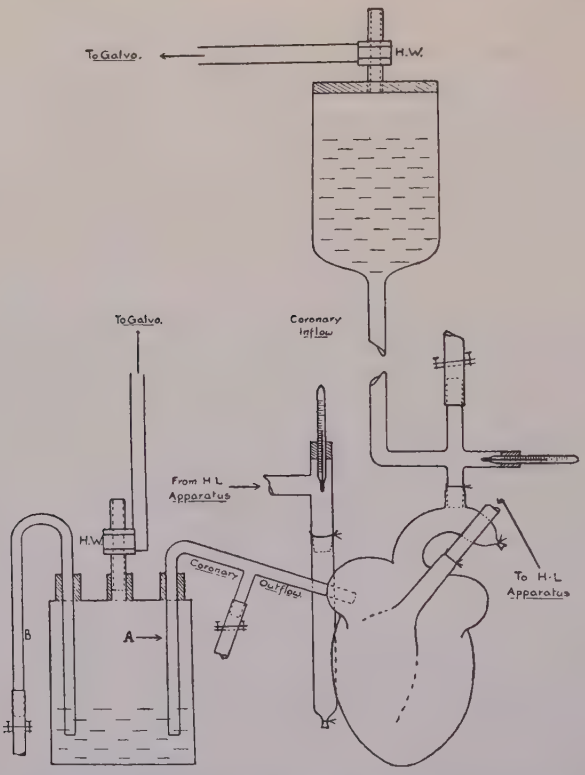

FIG. 5.—Arrangement of apparatus for recording outflow from and inflow into coronary circulation. (Anrep, Cruickshank, Downing and Suba Rau, *Heart*.)

As applied to the recording of coronary outflow, the arrangement shown at the left in Fig. 5 was employed. In this, the Morawitz cannula conveys the sinus blood into the Woulff's bottle, from which it can be periodically removed: the air displaced by its entrance flows over the

hot wire enclosed in the box HW. The whole outflow is taken as proportional to the sinus outflow.

For the study of the inflow into the coronary arteries, the warm (oxygenated) blood is contained in a reservoir placed at a suitable height to give the necessary pressure ; the hot wire is placed on the air-inlet into this reservoir, so that air flows over the wire to replace the blood which passes out

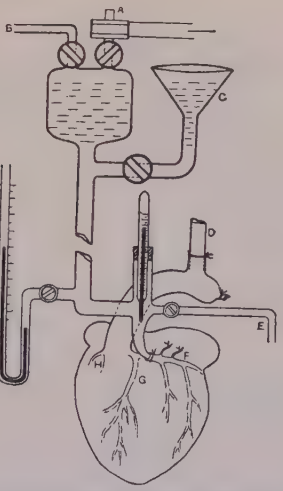

Fig. 6.—Arrangement for recording inflow into a branch of a coronary artery in the heart-lung preparation. A volume recorder is attached to B for giving a continuous record. A is the hot-wire chamber. (Anrep and Haüsler.)

from the reservoir through a rigid tube to the coronary arteries. The arrangement by which the blood is conveyed to the coronary arteries varies according to the requirements of the experiment. In some cases it is desirable to measure and record the whole arterial inflow, and the arrangement is then as shown (top right) in Fig. 5. In this type of experiment, the right ventricle, instead of pumping blood through the lungs, pumps it through a suitable resistance,

from which it returns again to the right auricle: the left ventricle is not doing any work.

Another arrangement for inflow is shown in Fig. 6. Here the heart-lung preparation is set up in the usual way, and the left ventricle can pump blood at a controlled rate and pressure; into a branch of the left coronary artery (*e.g.*,

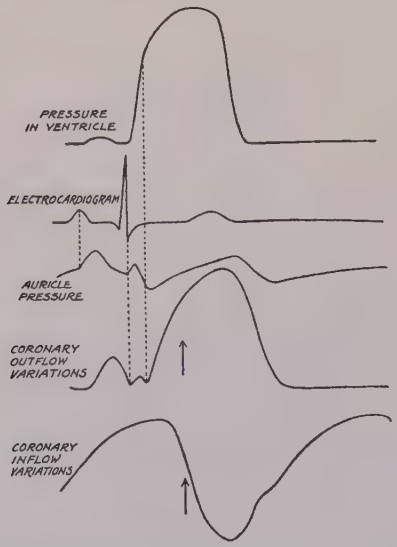

Fig. 7.—Relation of coronary outflow and inflow to other events of the cardiac cycle. The arrows point in the direction of increase of flow. (Anrep *et al.*, *Heart*, 1927.)

the circumflex) is inserted a cannula conveying the warm oxygenated blood from the inflow vessel.

This arrangement enables the chosen branch of the coronary artery to be perfused at any desired pressure, while the aortic pressure, and hence that of the remaining coronary branches, may be varied at will, as also may be the output of the heart. Together with these records, it was usual also to take records of some other variations, *e.g.*, of endocardic or aortic pressure, or of the electrocardiogram.

The general results of such experiments are illustrated graphically in Fig. 7. It will be seen that the sinus outflow shows three waves, very similar to those found in the curve of intra-auricular pressure. Of these, the first appears to be due to a sudden squeezing out of blood coincident with the auricular systole, and due to it, since it follows any alterations of auricular rhythm and disappears in auricular fibrillation.

The second wave is usually small, and coincides with the commencement of ventricular contraction, *i.e.*, with " *c* " of the auricular or venous pulse, (R) of the electrocardiogram, and with the steep ascent (isometric phase) of the intra-ventricular tracing ; its duration varies with the rate of the coronary circulation. It is followed by the large third wave, which begins with the period of ejection, reaches a maximum before the end of that phase, and then suddenly falls away.

These three waves are as well marked when the coronary artery pressure is kept constant, as in the experiments for studying inflow, as they are when the coronary artery pressure is subject to the normal fluctuations of arterial pressure. They are, therefore, in no sense due to the variations of coronary arterial pressure, but must be a result of the pressures exerted by the heart walls.

Investigation of the changes in the rate of inflow into the coronary arteries when these were perfused at constant pressures showed that there was a rapid entry of blood during diastole and early systole, which was suddenly checked as soon as ejection of blood from the left ventricle began, and reached its minimum at about the time that the coronary outflow was at its maximum, *i.e.*, at the height of the systolic reduction of volume.

The diminution of the rate of inflow began earlier when the coronary flow was large than when it was smaller, and is due to the pressure of the contracting ventricles upon the whole cardiac vasculature. In the case of the deeper vessels of the left ventricle, which during the height of systole are

exposed to a pressure greater than the systolic pressure in the aorta, there is probably complete cessation of flow into the arteries, and these, together with the capillaries and veins, are subjected to the powerful pressure of the ventricle, by which the blood is swept out, mainly through the veins, as the outflow records indicate.

In the conditions of the experiments by the method illustrated in Fig. 6, however, it is possible, when desired, to have the selected arteries perfused with blood at a pressure much below that in the ventricle and aorta, and, in this case, the blood may, during systole, flow *backwards* along that branch supplied at the low pressure. This is because blood enters the arteries, not only during diastole, but also through collateral vessels, from the coronary branches at a higher pressure during systole, and flows in the reverse direction along the arteries under lower pressure. It is quite conceivable that if, under normal conditions of supply, there was a partial obstruction to one of the main coronary branches, a similar state of affairs might ensue, and result in the maintenance of a circulation of blood in a part which otherwise might be insufficiently fed.

From these experiments it was shown that the coronary circulation, always and only restricted during systole, became smaller and smaller the higher the arterial, and, therefore, the endocardiac, pressure became; analogous experiments in which the cardiac output per beat was altered showed that the effect was relatively slight, but that with very great outputs there was a slight diminution in the coronary supply. The following table illustrates this. It must be remembered that the work of the heart varies approximately directly as the arterial pressure, but as the square of the output.

In these experiments we see the pure effect of the altered force of the cardiac contraction, while the coronary perfusion pressure is kept constant. Under conditions in the body, this factor is, of course, never dissociated from another most

important one, namely, the concomitant alteration in the coronary artery pressure. We shall see that when the heart performs more work this last factor, together with others, more than compensates for the reduction of flow due to pressure of the contracting myocardium.

The coronary inflow does not reach its maximum immediately diastole sets in, as might have been expected, but rises

Effect of Change of Arterial Pressure and Cardiac Output on Coronary Blood Flow. (Anrep and Haüsler.)

Coronary Perfusion Pressure mm. Hg.	Mean Aortic Blood Pressure. mm. Hg.	Systemic Output c.c. per minute.	Inflow into Coronary Artery, c.c. per minute.
115	40	—	25
115	60	—	20·6
115	110	—	16·0
115	140	—	13·5
84	80	250	33·4
84	80	500	31·4
84	80	1,400	25·5

steadily to a maximum at about the commencement of the next systole. The delay is more marked at low coronary pressures, and is explained by Anrep and Haüsler as due to a viscous resistance in the coronary vessels, which causes them only slowly to open up again under pressure.

The Effect of Arterial Pressure on Coronary Flow

As in other parts of the body, the rate at which blood flows through the coronary vessels must largely depend upon the pressure at which it is supplied, and it has long been known from experiment that this is so. Indeed, in the

ordinary or denervated heart-lung preparation, the arterial pressure at the moment is the only important factor. The following experiment of Markwalder and Starling on a heart-lung preparation is an illustration.

Arterial B.P. mm. Hg.	Total Coronary Flow, c.c. per min.
20	24
40	45
60	84
80	108
100	147

At the lower pressures the coronary flow is very small, and may even be inadequate to supply the heart properly, but it rapidly increases with rise of arterial pressure. Under normal conditions Evans and Starling estimated the coronary flow as about 40 c.c. per gram of heart per hour. Anrep, in more recent experiments, considers it to be from 40 to 60 c.c. per gram of heart per hour, but the figure is, in any case, subject to wide variations.

The interesting question arises in connection with the relation of arterial pressure to coronary blood supply as to which phase of the arterial pressure cycle is decisive for the rate of blood flow. The practical importance of this is in clinical conditions in which the pulse pressure changes without much alteration in the mean pressure, *e.g.*, in aortic regurgitation, or in consequence of changes in the relation between output and peripheral resistance.

The systolic phase obviously cannot be the one concerned, because, as we have seen, although the arterial pressure is then at its height, the endocardiac pressure is higher still, and thus retards or stops the inflow. The important phase must be the one when there is the greatest excess of aortic

over endocardiac pressure, which is some time during diastole; this will not necessarily be at the "diastolic pressure" at which phase the arterial pressure is at its lowest, though Smith, Miller and Graber are of the opinion that the coronary flow does really depend on the diastolic pressure.

Anrep and King have reinvestigated this question on the heart-lung preparation, taking optical records of aortic pressure and observations of coronary sinus flow. The following variables were involved: heart rate, output, systolic pressure, diastolic pressure, coronary flow; the rate could be controlled by electric stimulation of the auricle, the output by varying the inflow, the systolic and diastolic

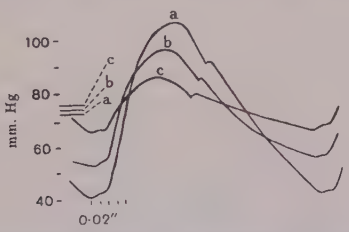

FIG. 8.—Superposed aortic pressure records under following conditions:—

	a	*b*	*c*
Output, c.c. per min.	960	520	250 (varied)
Coronary sinus flow, c.c. per min.	37·5	37·5	37·5 (kept constant)
Arithmetical average B.P., mm. Hg.	73·0	74·5	76·0

(Anrep and King.)

pressures by suitable adjustment of the elastic artificial resistance and of the rate of beat. They varied their experiments so as to alter the output or the rate, but main-

tain constant either: (1) The systolic pressure; (2) the diastolic pressure; or (3) the coronary flow, the aortic pressure changes being recorded in each case.

They found that when the output was changed, the coronary flow did not remain constant when either the systolic or the diastolic pressure was kept uniform, but only when the mean aortic pressure remained the same. Fig. 8 shows superimposed tracings of the aortic pressure in an experiment in which the output was altered, but the coronary flow kept constant by altering the systemic resistance. It will be seen that the mean arterial pressures are approximately constant, but that the systolic and diastolic pressures vary greatly.

When the rate of the heart is altered the same relations again hold good, *i.e.*, provided the mean aortic pressure is kept constant, the coronary blood flow remains constant (Fig. 9).

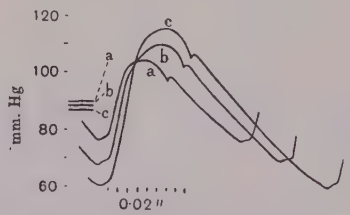

Fig. 9.—Superposed aortic pressure tracing from an experiment in which the heart rate was altered.

	a	*b*	*c*
Heart rate, per min. . . .	125	150	188
Coronary flow, c.c. per min. . .	39	39	39

(Anrep and King.)

Changes in the systemic output and in the heart rate have therefore no effect on the coronary blood flow in the *de-*

nervated heart except in so far as they alter the mean aortic pressure.

According to Nakagawa, Hilton and Eichholtz, and Hammouda and Kinosita, fibrillation of the ventricle or its arrest in diastole is accompanied by an increased coronary flow, while arrest in systole causes a diminution ; Sharpey-Schafer and Wiggers, however, believe that the coronary flow is greatly assisted by the massaging action of the cardiac contractions.

Relation of Coronary Flow to Pulmonary Circulation

It is easy, with the heart-lung preparation, to show that, as might be anticipated, when the conditions of inflow from the venous reservoir into the heart are kept constant, small alterations in the aortic pressure cause but little change in the rate at which blood is pumped out on the arterial side through the artificial systemic resistance. Calling this rate of flow the systemic output, it can easily be shown, however, that this is not absolutely constant, but diminishes if the arterial resistance is much increased.

This apparent falling-off in the systemic output is due to the fact that, as the arterial pressure is raised, more and more of the blood ejected from the left ventricle is passed along the coronary circulation and so short-circuited back to the right side. When the coronary circulation is allowed for, Markwalder and Starling found that the total output of the left ventricle is much more nearly constant than would appear from measurement of the systemic output, as the data on p. 20 show. It is seen that, except for very high pressures with large outputs at which the heart begins to fail, the total output remains fairly steady.

In these experiments, in which the Morawitz cannula was used, some three-fifths of the total coronary flow has been passed outside the heart altogether, but with the intact heart the whole, or nearly the whole, coronary flow would be

returned to the right side and added to the normal inflow into, and therefore output from, the right ventricle. A modification of the heart-lung circulation has been made by Dusser de Barenne, which enables the whole coronary blood flow to be collected with the use of only one heart. It will

Systemic and Total Output of Heart. (Markwalder and Starling.)

Dog's heart, weight = 107 grams. Rate = 150 per minute
Constant Inflow.

Arterial Pressure, mm. Hg.	Systemic Output, c.c. per min.	Total Coronary Flow, c.c. per min.	Total Output, c.c. per min.
44	790	30	820
84	811	41	852
108	790	50	840
140	770	71	841
170	750	117	867
208	600	260	860
60	1,402	50	1,452
100	1,260	90	1,350
128	1,222	125	1,347
166	1,178	208	1,386
190	710	500	1,210

be seen from Fig. 10 that this differs from Starling's heart-lung preparation by having the venous reservoir supply the peripheral end of the pulmonary artery direct. All the blood from the coronary flow therefore drains into the heart and may be collected by a cannula tied into the superior vena cava. With this preparation the dependence of coronary flow on systemic blood pressure is very clearly shown. The following is one of de Barenne's experiments :—

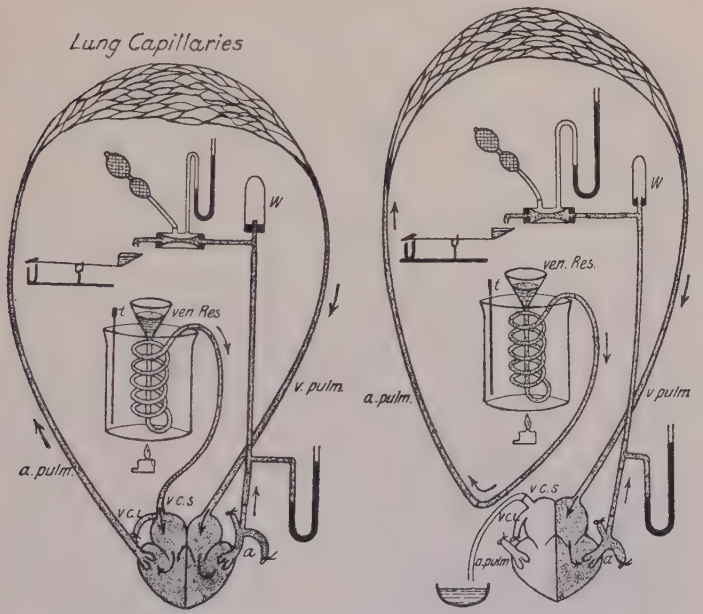

FIG. 10.—On the left, scheme of Starling's heart-lung preparation. On the right, de Barenne's modification. (Dusser de Barenne.)

Dog's heart, 36 grams. Pulmonary Pressure kept Constant at about 41 mm. Mercury

Arterial Blood Pressure, mm. Hg.	Systemic Output, c.c. per min.	Total Coronary Flow, c.c. per minute.	Total Output of Left Ventricle, c.c. per minute.
81	194·9	21·8	216·7
110	191·0	28·0	219·0
141	176·5	43·0	219·5
163	155·5	53·1	208·5
184	123·2	67·0	190·2
201	107·3	84·2	191·5
227	67·8	99·2	167·0

It will be seen that with a blood pressure of 227 mm., the systemic output from the left ventricle is actually less than (0·68 of) the coronary flow. Here again the total output of the left ventricle is approximately constant until very high pressures are reached, when it falls off owing to failure of the left ventricle.

We should expect to find, therefore, that in the intact heart there would be an actual *increase* in the output of both ventricles when the arterial resistance was raised, because to the constant inflow would be added the increment to the coronary flow, brought about by the increased arterial resistance. Experiment shows that this is so, and provides further interest in connection with the relation between pulmonary and systemic pressures. Some observers (*e.g.*, Fühner and Starling, Straub), but not all, have found that the pulmonary artery pressure rises and falls with the aortic pressure, and it has been supposed by some that this correspondence might be due to what has been called "back pressure," *i.e.*, to incomplete emptying of the left ventricle, with consequent distension of the left auricle and increase of pressure throughout the pulmonary system. Back pressure effects of this type undoubtedly play a part in pathological phenomena, when there is weakness of the left ventricle or deficiency of the mitral valves. Actual observation showed, however, that in healthy hearts there was no invariable increase of pressure in the left auricle when the aortic pressure was raised to any reasonable extent—there might even be a fall in pressure, or, if the output was also increased, there might be a rise, often a transitory one : most usually there is but little effect. A rise of left auricular pressure is more easily produced by an increase in the output of the heart, and such changes as are seen on moderate increase of arterial resistance are quite possibly due to this increased circulation of coronary blood by which the output of the ventricles is augmented. In any case, the change in the left auricular pressure is always slight compared with that in the

pulmonary artery, so that back pressure cannot explain the concomitant changes in pulmonary pressure. Anrep and Bulatao showed that if the coronary circuit was excluded, as it was partially when a coronary cannula was inserted or completely by the use of de Barenne's heart-lung preparation, moderate increase of the aortic resistance was without influence upon the pulmonary pressure : very great increase did cause a sudden rise, but this only happened when there was such dilatation and inefficient emptying of the left ventricle as to cause a distension of the left auricle and a real back pressure effect. Here is one of their experiments :—

Heart-lung Preparation. Output between 560 and 590 c.c. per Minute

Before de Barenne's Modification

Systemic B.P., mm. Hg.	60	120	60	150	40
Pulmonary B.P., mm. water	190	240	200	270	190
Left auricular B.P. . .	25	28	25	20	28

After the Establishment of de Barenne's Modification

Systemic B.P., mm. Hg. .	60	100	130	150	60	40	180	40
Pulmonary B.P., mm. water .	200	200	196	196	200	200	190	200
Left auricular B.P. . .	28	20	12	—	25	30	0	28

They further proved this by measuring the total flow along the pulmonary artery with a strohmuhr ; this was greater than the systemic output by an amount equal to the coronary flow. When the aortic pressure was raised, the pulmonary flow was increased, the systemic flow was either the same, or was reduced if the rise of aortic pressure was great. Hence it would appear that the changes in pulmonary pressure

which accompany alteration in aortic pressure are owing to increased output of both ventricles due to the coronary short circuit.

Influence of Various Chemical and Physical Factors on the Coronary Circulation

The influence of various chemical and physical agencies may be studied directly on the coronary circulation by one of the methods already described, but valuable information is also given by the study of isolated coronary arteries. Some of these will now be considered. As Haüsler has shown, however, the effect of various conditions on the coronary vessels cannot be directly inferred by measuring the alterations in coronary blood flow, because, in addition to any effect which these agents may produce on the coronary vessels, the rate of coronary flow will be greatly affected by alterations which may result in the strength, frequency or duration of systole. Small doses of adrenaline, for instance, by affecting the force of systole to a greater extent than the calibre of the coronary vessels, may produce apparent vasoconstrictor effects. From his experiments, it would seem that the safest criterion of action on the coronary vessels is the determination of the rate of coronary inflow in the artificially driven heart during the diastolic period, provided that in all cases the inflow is stopped during systole.

The Effect of Temperature.—Recent experiments by Cruickshank and Subba Rau show that in some respects the larger branches of the coronary arteries behave differently from branches of the systemic arteries. When removed from the body the latter are always in a state of contraction, whereas the coronary arteries are relaxed. On warming up, the coronary artery contracts steadily from 22° C. to about 38° C. From 38° to 45° there is a slight relaxation. Systemic arteries show first a slight contraction, and then, with further rise of temperature, show steady relaxation up to about 45° C.

Effects of Hydrogen-ion Concentration.—Increase of *p*H (lowering of H-ion concentration) causes contraction of the artery particularly between *p*H 7·5 and *p*H 7·9. Reduction of *p*H below *p*H 6 also causes contraction.

The contraction of the coronary arteries with rise of *p*H is of importance in connection with the perfusion of hearts with fluids containing bicarbonate. It was shown by Dale and Evans that a heart can be satisfactorily perfused with

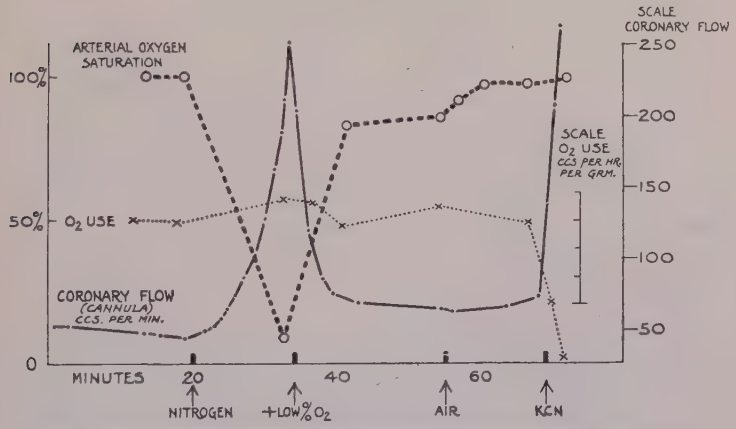

FIG. 11.—Curves showing the relation of the coronary blood flow, and of the oxygen consumption by the heart to the degree of oxygen saturation of the blood passing through the coronary vessels. (Hilton and Eichholtz.)

Ringer's solution containing as much as 0·2 per cent. of $NaHCO_3$, provided that it is saturated with oxygen containing sufficient carbon dioxide to prevent the *p*H of the fluid from rising much above 7·5.

Effect of Oxygen.—In the presence of oxygen, rings made from the coronary artery show rhythmic contractions similar to those which are seen in the systemic arteries. These disappear in the absence of oxygen. The relaxed condition of the coronary arteries at lower temperatures is not altered by the presence of oxygen, but when warmed up the con-

traction which is otherwise seen remains in abeyance if oxygen is withheld.

It has long been recognised that asphyxiation of the heart produces very large increases in the coronary flow (see Table, p. 7). These may be in part due to the relaxing effect of the absence of oxygen. Hilton and Eichholtz found that the degree of dilatation of the coronary vessels was almost proportional to the degree to which the arterial blood had become desaturated (see Fig. 11). The heart muscle, as shown by Evans and Starling, is able, in conditions of asphyxia, to abstract oxygen almost completely from the blood. It appears from the experiments of Hilton and Eichholtz that the increased rate of coronary circulation is in some way so adjusted as to supply the heart with its normal amount of oxygen in spite of the deoxygenation of the arterial blood (see Fig. 11). The same great dilatation of the coronary vessels is seen when traces of cyanide are added to the blood so as to stop the oxygen utilisation of the tissues.

This effect is to some extent to be attributed to the action of oxygen lack on the plain muscle in the walls of the coronary artery; it has been shown by Garry that plain muscle, in general, is relaxed by lack of oxygen, though to an extent which differs according to the source of the muscle.

Perhaps, in addition to the action of anoxæmia, there may be an effect due to the accumulation in the tissues of metabolites removable, or partially removable, by oxygen. If this is the case, these metabolites apparently tend to increase in amount in a heart-lung preparation, so that towards the end of an experiment the coronary flow is found to be very much greater than it was at the beginning. The nature of these metabolites is quite unknown. On the other hand, certain facts are opposed to the increase of coronary flow in long experiments being due to the accumulation of metabolites in the blood; for example, Hilton and Eichholtz found that, if at the end of a long period the blood was

withdrawn and replaced by fresh blood, no return of the coronary flow to its former level took place.

Increase of the carbon dioxide content of the blood can also cause some dilation of the coronary vessels, but the extent of this increase is small compared with the effects of adrenalin or oxygen lack.

Other Substances.—Adrenaline, which is known directly to augment the coronary circulation (see p. 24), also causes relaxation of the smaller branches of the isolated coronary arteries. The larger arteries, on the other hand, behave like systemic arteries and show constriction with adrenaline. The dilator action of adrenaline on the smaller branches is not abolished by previous administration of ergotoxin, but the constrictor action on the larger branches is so abolished. Histamine causes contraction of the coronary arteries of the ox, rabbit and man, but dilates the vessels, particularly the smaller ones, in the cat.

Nervous Control of the Coronary Circulation

Up to the present we have discussed chiefly the coronary circulation in the heart-lung preparation made in the usual way, and therefore denervated. The coronary arteries, however, as has been shown by Woollard, are richly innervated from both the vagus and sympathetic, branches of which follow the coronary arteries up to their smallest ramifications. The innervation of the smallest branches is mainly from the vagus (Fig. 12), that of the larger branches from both sources.

Various efforts have been made in the past to ascertain the influence of the nerve supply on the coronary blood vessels. On the whole, the opinion of workers, *e.g.*, Wiggers, was that the vagus carried constrictor fibres. This has been confirmed by Anrep and Segall by experiments on the innervated heart-lung preparation. In this arrangement there is a heart-lung preparation of the usual type, and, in

addition, a pump for supplying the head of the animal with blood. They found that the vagal fibres are normally in action and so render the coronary flow smaller than it would be in the denervated preparation. Section of the vagus therefore causes relaxation of the coronary vessels by releasing these from the vagus tone, just as it allows quickening of the heart rate.

FIG. 12.—Innervation of a minute branch of the coronary artery from a cat with both stellate ganglia removed. The perivascular plexus must therefore be largely vagal. (Woollard.)

The increase in coronary flow which results from section of the vagus is, however, not due to the concomitant acceleration of the heart rate ; the increase in flow persists, while the rate of heat remains constant, if atropine has previously been administered, or if the heart is driven electrically at a suitable high rate : it has also been shown independently that the coronary circulation is not appreciably affected by the rate of the heart beat.

An important reflex was found by Anrep and Segall to be operative when, in the innervated heart-lung preparation, the output of the preparation was raised. We have seen that, with the denervated preparation, increase of systolic output has but little effect on the coronary flow or even causes a slight reduction, but when the nerve supply is intact, an increased output is followed by an increase in the coronary flow. This effect is lost on section of the vagi. In the intact animal, therefore, when the output of the heart is increased there is, in addition to the reflex cardiac acceleration by the Bainbridge reflex, also an independent reflex relaxation of the coronary flow whereby the heart receives an increased blood supply to enable it to perform its extra work.

What the effect of the sympathetic supply to the heart may be is not so certain, but since adrenaline produces such a large increase in the coronary flow, and since an increase of coronary flow also follows stimulation of the stellate ganglia, it seems reasonable to suppose that the sympathetic supply has, on the whole, a vaso-dilator effect, particularly on the smaller branches of the coronary system.

Stimulation of various sensory nerves, or cerebral anæmia, also causes considerable coronary vaso-dilatation, which is prevented by extirpation of the stellate ganglia.

To sum up, therefore, we see that the coronary circulation is dependent upon various modifying actions. First and foremost upon the arterial pressure, next upon its chemical surroundings ; and, thirdly, upon the reflex control of a tone imposed upon it through the nervous system. The mechanisms which adjust the coronary flow to the work which the heart has to perform, or to the conditions in which it is placed, are therefore elaborate and capable of great latitude in their effects.

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CHAPTER II

THE PART PLAYED BY THE CAROTID SINUS IN THE REGULATION OF THE CIRCULATION

Among the various reflexes which control the circulation by bringing about alterations in the rate of the heart or in the vasomotor tone, physiologists have long recognised the depressor mechanism which was described in 1866 by Ludwig and Cyon. Daly and Verney and Anrep and Segall produced definite evidence that rise of pressure in the aorta could act as an adequate stimulus to this afferent supply, causing reflex cardiac slowing and vascular relaxation. Within recent years, however, another important mechanism has been revealed through the researches of a number of physiologists, prominent amongst whom are H. E. Hering and C. Heymans, and their collaborators. These investigations have shown the existence of an important zone in the neighbourhood of the bifurcation of the common carotid artery, by which heart rate and vasomotor tone are reflexly controlled, in response to various stimuli, conspicuously to those due to alterations in the degree of distension of the walls of the blood vessels in this region.

This discovery had been in a measure adumbrated by sundry earlier and hitherto unexplained observations : thus, the occlusion of both carotid arteries was known to produce acceleration of the heart and rise of systemic arterial blood pressure : increase of pressure in the blood vessels of the head produces slowing, and fall of blood pressure acceleration of the heart ; traction upon the cephalic end of one carotid artery produces a fall of arterial blood pressure, with cardio-inhibition (Sollman and Brown). Further, there was

the experiment of Tschermak, who, in 1866, showed that compression of the neck high up in the region of the vagus, in man, caused slowing of the heart. In 1923, H. E. Hering showed that this fall of blood pressure with cardiac slowing was due, not to mechanical excitation of the vagus, as

FIG. 13.—Showing carotid sinus in man: (1) common carotid; (2) carotid sinus; (3) internal carotid; (4) external carotid; (5) nerve to carotid sinus; (6) glossopharyngeal nerve. (C. Heymans, "Le sinus carotidien.")

Tschermak had supposed, but to a reflex from the carotid sinus, as this region is now called.

The Carotid Sinus.—The carotid sinus, in the physiological sense, signifies a specially innervated part of the vessels and tissues in the neighbourhood of the bifurcation of the common carotid into its branches, and includes also the carotid body. In man (see Fig. 13), and many other mammals, the common carotid artery divides into the internal and external carotids, and a bulbous expansion, the

carotid sinus or carotid bulb, is seen at the root of the internal branch. In the ox, the common carotid divides into three branches, the external carotid, the glosso-facial and the occipital arteries. The swelling corresponding to the sinus caroticus in man is found at the root of the occipital artery in these animals.

A distinct nerve twig runs from the carotid sinus to the glossopharyngeal. These nerves originate in the carotid sinus as a fine network of fibres in the adventitial coat of the artery, while some of them also are connected with definite bulbous endings (see Fig. 14). A small branch which passes

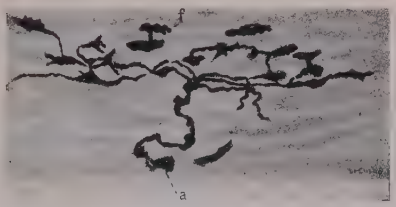

FIG. 14.—Sensory endings in the adventitia of the carotid sinus in man: (*a*) arborising nerve fibres; (*f*) end organs. (C. Heymans, *after* de Castro.)

from the carotid sinus to the superior cervical ganglion has also been described by Hering and by Danielopolu.

The carotid body, which was formerly supposed to be an endocrine organ, is believed by de Castro and by Heymans to be related in some way to the carotid sinus. This organ is very richly supplied with blood vessels, there being a special arteriole and venule to each clump of cells, and sinusoids freely distributed amongst them. Around the arteries is a network of nerves derived from filaments which run to the glossopharyngeal and to a small extent to the ganglion of the vagus trunk. All the fibres of the carotid sinus region are afferent in function, so far as has been ascertained.

Fig. 15 illustrates diagrammatically the afferent nerve

supply responsible for reflex control of the circulation in response to arterial pressure changes, *i.e.*, the depressor and carotid sinus innervation. The efferent paths are, of course, the vagus, for cardio-inhibition, and the sympathetic nervous system, connected with the relevant bulbar centres by way of the spinal cord, for vascular tone and cardio-acceleration.

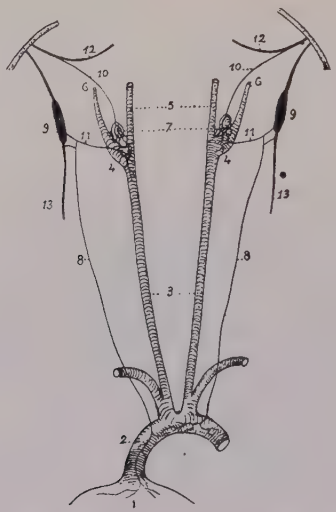

FIG. 15.—Scheme of the sensory innervation of the carotid sinus, carotid body and arch of the aorta. (1) Heart; (2) arch of aorta; (3) common carotid; (4) carotid sinus; (5) external carotid; (6) internal carotid; (7) carotid bodies; (8) depressor nerve; (9) ganglion of vagus; (10) branch to carotid sinus joining the glossopharyngeal; (11) branch to carotid sinus joining vagus ganglion; (12) glossopharyngeal; (13) vagus. (C. Heymans.)

Reflex Function of the Carotid Sinus.—Stimulation of the carotid sinus electrically, or mechanically, as by stretching its walls by pressure from within in any manner, provokes a combined reflex of cardiac inhibition and fall of blood pressure, just as does stimulation of the central end of the depressor nerve. In fact, we may regard the reflexes from the carotid sinus and those from the depressor nerve as part and parcel of the same physiological arrangement for con-

trolling the blood pressure. Florey and Marvin have shown that this depressor reflex on stimulation of the carotid sinus is particularly well seen in rabbits anæsthetised with urethane. As with the depressor nerve, it is found that, after section of the vagus in the neck, stimulation of the carotid sinus in any way no longer produces the cardiac inhibition (proving that this was reflex), but will still provoke a fall of blood pressure. By the application of ingenious methods of experimentation, the various reflexes in which the carotid sinus is concerned have been analysed by C. Heymans and his collaborators, and some of these experiments will now be considered.

"Perfusion" of the Isolated Carotid Sinus.—One of the most striking experiments of Heymans is that called the "perfusion of the carotid sinus." For this purpose, in a dog (B), one of the common carotids and also all the branches springing from just beyond the bifurcation are tied off, so that the sinus region is isolated from the circulation and left connected with the animal only by its nerves; this isolated segment of carotid artery with its branches can then be perfused with blood from a second dog (A), with the carotid artery and external jugular vein of which the carotid sinus of dog B has been put into communication. The blood from the carotid of A enters the cephalic end of the carotid artery of B, and, after passing along the carotid sinus segment is returned *viâ* the external carotid artery of B to the external jugular vein of A (Fig. 16). Connection between the vessels is made by direct vessel-to-vessel anastomosis, so that no anti-coagulant is required to prevent the blood from clotting. In effecting this anastomosis, Payr's tubes are used; the smaller vessel is passed through the tube, and its end everted to form a cuff, with the intima on the outside, over the end of the tube. The larger vessel is then pulled over this cuff, so that the intimas of the two vessels lie in contact. The blood is thus always in contact with nothing but vessel intima.

Various procedures with this preparation illustrate the reflexes which originate in the carotid sinus. The following is typical: the preparation being established, clips are placed on the connecting vessels, first on the artery, then on the

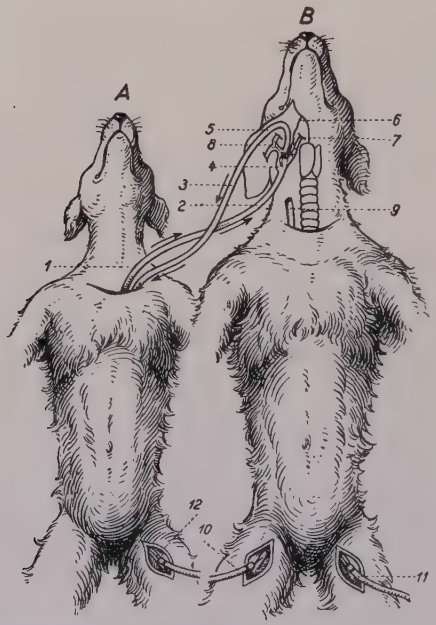

FIG. 16.—Perfusion of isolated carotid sinus. A, dog supplying perfusion blood. B, dog of which the sinus is perfused. 1, Carotid artery of dog A. 3, External jugular of dog A. 6, Nerve supply of carotid sinus of B. All the branches of the carotid sinus of B are tied off, so that the only blood entering it is that from A. (C. Heymans.)

vein, so that no blood is flowing to the isolated sinus, and the pressure in this is low. If now the clips are opened, so that blood, at the pressure prevailing in dog A, circulates through the sinus of dog B, there is an immediate fall of arterial blood pressure in the systemic circulation of the latter. If the blood pressure of A is still further increased,

for example, by clipping off its own right carotid and therefore raising the pressure in its left carotid, there is a still further fall of pressure in dog B (Fig. 17). If the blood pressure tracing of dog A shows extensive Traube-Hering waves, then similar waves, in the opposite phases, will

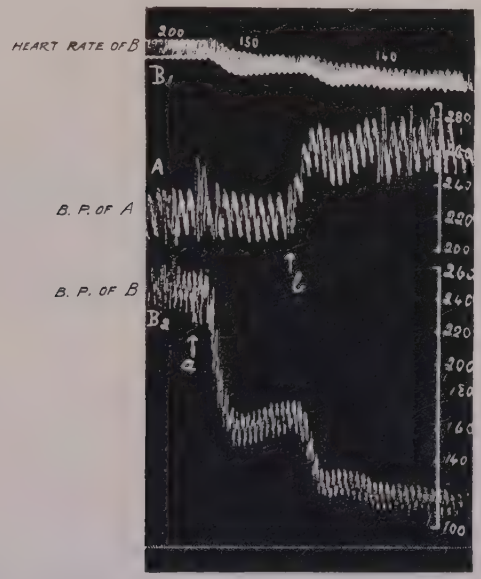

FIG. 17.—Preparation made as in Fig. 16. Tracing B_1, record of heart rate of dog B. Tracing A, blood pressure of A, Tracing B_2, femoral blood pressure of dog B. On allowing blood from A to flow into the sinus of B at the point *a*, there is a fall of pressure and cardio-inhibition in B. At *b*, pressure in A is raised by clipping off right carotid artery. Still further fall of pressure and cardiac slowing in B. (C. Heymans.)

accompany them in dog B. Together with the fall of pressure, there is a slowing of the heart in B, and, conversely, if by lowering the pressure in the carotid sinus of B, its systemic arterial pressure is raised, there is at the same time cardiac acceleration in B (Fig. 17). The alterations of pressure, though they accompany the alterations in heart

rate, are not due, or are only in part due, to them, since the pressure changes still persist after elimination of the cardiac effects by section of the vagi or administration of atropine.

It is found that the slowing of the heart is due to a unilateral reflex. This can be shown if, for example, as in Fig. 16, the right vagus of dog B is divided. Changes of blood pressure brought about in B as a result of alterations of pressure in A are then not accompanied by changes in heart rate.

It is probable that in these reflex alterations of frequency there is some intervention on the part of the sympathetic supply, though there seems no doubt that the main effects are due to alterations of vagus tone.

Effects of Changes in Cephalic Arterial Pressure

The most important question which arises out of the discovery of the carotid reflexes is that which concerns the effect on the general circulation of increased arterial pressure in the head. Various investigators have studied this question, but with divergent results. It has been generally agreed that an increase in the intracranial arterial pressure tends to produce, as compensatory phenomena, slowing of the heart and a fall in the somatic blood pressure. There was uncertainty, however, as to whether this fall was entirely due to the operation of cardiac factors, or whether vascular changes were also involved. It was necessary, in order to solve this question, to have a means of studying the cranial and somatic circulations separately. This was accomplished by Hédon, who, by a method of cross-circulation, found that both mechanisms were involved, and Heymans and Tournard, Chabrol and Marchand, and others have improved upon his technique and amply confirmed and extended his original observations. It was concluded from these experiments that rise of pressure in the head produces fall of pressure in the body by means of both cardio-inhibition and vasomotor relaxation, and we now know that the

reflexes from the carotid sinuses must play a large part in causing this response.

The next question that arose, however, was whether or to what extent these reactions might be the result of direct

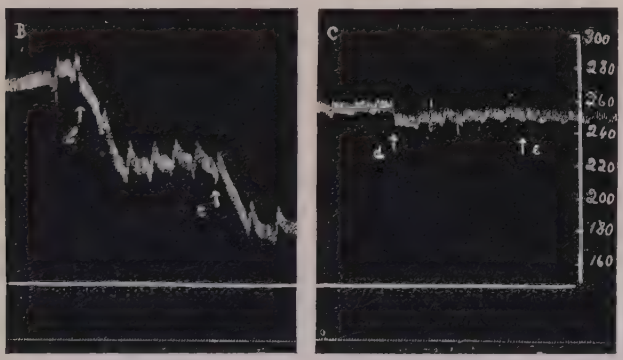

FIG. 18.—Showing the effect of carotid sinus reflexes on blood pressure in an intact anæsthetised dog—the record shows femoral blood pressure. B: the carotids are clipped off; at *b*, one clip is removed; at *c*, the second clip is removed. The blood pressure falls from 280–210, and then to 180 mm. mercury and the heart becomes slower. C: after denervation of the carotid regions, removal of the first clip at *d*, and second clip at *e*—no effect. (C. Heymans.)

effects of the altered pressure upon the cardio-inhibitory and vasomotor centres respectively. Most investigators until recently came to the conclusion that the effects were wholly direct, until it was shown by Hering that these effects of raised intracranial arterial pressure disappeared when all the nerves in the neighbourhood of the carotid sinus had been destroyed. This can be illustrated by the simple experiment of lowering the cranial pressure by occluding the carotid arteries (Fig. 18). It will be seen that the effects disappear if the carotid sinus is denervated.

Hering's conclusion that this zone therefore represented an important reflexogenic area for regulating cardio-vagal and vasomotor tone has been fully confirmed, especially by

Heymans, but his further claim that rise of pressure in the arteries of the head produces its effects exclusively in that manner, and never by exerting a direct effect upon the bulbar centres, is perhaps not so generally accepted. That the medullary centres which are concerned in regulating the circulation are liable to be directly affected by alterations of

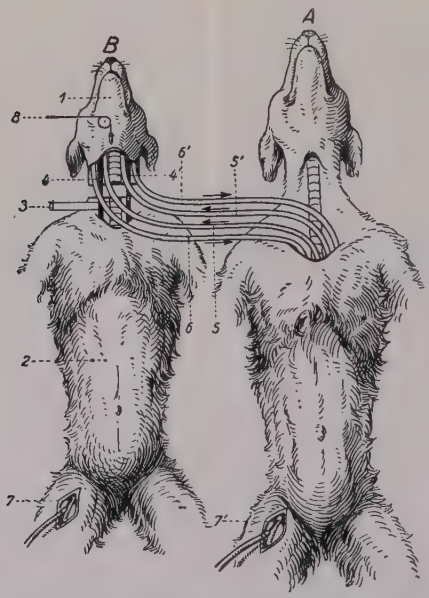

FIG. 19.—Schema of the isolated head, according to J. F. Heymans. The isolated head of B is supplied with blood from dog A, and is left in connection with its own body only by the two vagus nerves. (C. Heymans.)

intracranial pressure was held to be true by Anrep and Segall, and by Nash, and also by Anrep and Starling, but has been denied by Florey, Marvin and Drury.

An application of the technique of the " isolated head," as described by J. F. Heymans and his collaborators, enables this question to be investigated. In this preparation (Fig. 19) the head of dog B is fed exclusively from dog A by

anastomoses between the carotids and external jugulars of the two animals—all the tissues of the neck of B, with the exception of the two vagi, being divided. The head of B, therefore, is in vascular connection with A, and its only connection with its own body is through the two vagus nerves.

With this preparation it can be shown that a rise of pressure in A leads to a slowing of the heart in B. If the carotid sinuses of B are denervated, alterations of pressure in A no longer produce any effects upon the heart rate of B. We must conclude, therefore, that pressure changes in the head produce their effect upon the heart rate, not through any direct action on the cardio-inhibitory centre, but entirely through reflexes generated from the carotid sinus.

The vasomotor reflexes which are originated from the carotid sinus may be investigated by a somewhat similar technique. Here it is necessary to divide the vagi of dog B, but to leave its spinal cord intact, so that the head of dog B is connected with its body through its spinal cord, but receives all its blood supply from dog A. We may call this the "semi-isolated head." It is found that with this preparation, alterations in the arterial pressure in A produce compensatory effects in the opposite direction in the arterial pressure in B, provided that the innervation of the carotid sinuses of B is intact. These changes are not necessarily accompanied by alteration in heart rate. If the nerves to the carotid sinuses of B are divided, the reflexes disappear.

Site of the Effector Agents in the Vasomotor Reflexes.—The site of the effects involved in the vasomotor reflexes in B can be investigated by further modifications of the technique described above. It is found that the chief sites of vasoconstriction or vasodilatation are in the abdominal organs. The technique of the perfused kidney may be taken as an example illustrating this. In this experiment the kidney of dog B is perfused from one carotid and jugular of dog A, its nervous connections being left intact (Fig. 20).

The kidney of B is placed in a plethysmograph and its volume recorded. One of the carotid sinuses of B, say, the left one, is denervated, and the other retains its normal nervous connections. On clipping off the right carotid of B (normally innervated), there is a rise of pressure in B's systemic circulation. This is accompanied by a constriction of the kidney.

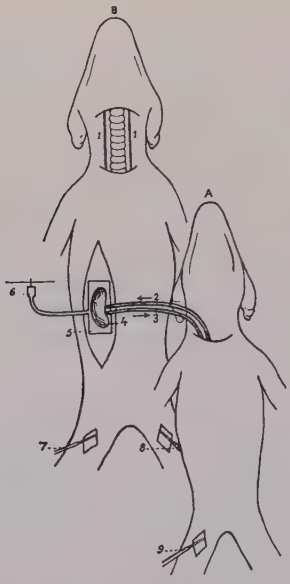

FIG. 20.—Showing the technique of the isolated kidney of dog B, which is perfused from dog A. (C. Heymans.)

When the denervated sinus is similarly treated by occluding the left carotid instead of the right, there is no effect on the blood pressure or kidney volume of B. The efferent path concerned is mainly the splanchnics.

Some experiments of Heymans and Bouckaert indicate that the vessels of the head itself also share in these changes in the vasomotor response, and in the same direction as those of the viscera.

Reflex Secretion of Adrenaline.—Another important effect is that the reflexes involving vasoconstriction are also accompanied by an output of adrenaline from the suprarenals, and those involving vasodilatation with a reduction

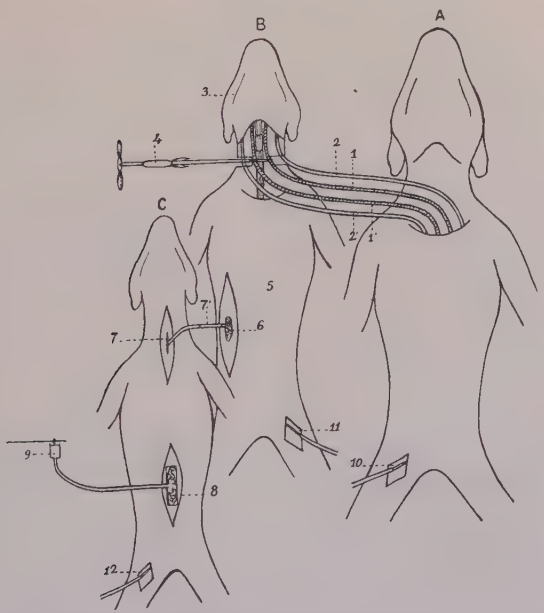

Fig. 21.—Perfusion by carotid and jugular anastomosis from A of semi-isolated head of B (right carotid sinus denervated), and from suprarenal vein of B to jugular of C (epinephrectomised). Spleen of C in plethysmograph contracts when adrenaline is liberated in increased amount from B's suprarenals, as, *e.g.*, when pressure in left carotid (innervated) of B is lowered. Systemic pressures of each dog are recorded from femoral artery. 4 = Chassaignac's écraseur, dividing neck tissues of B, except spinal cord. Vagi of B divided. (C. Heymans.)

of adrenaline output. This can be demonstrated by a slight modification of the crossed circulation technique, as used by Tournade and Chabrol in their suprarenal-jugular anastomosis. One arrangement of this experiment as carried out by Heymans is shown in Fig. 21.

This consists of dog A, the feeder, supplying the semi-isolated head of dog B, *i.e.*, the head connected with its own body only by the spinal cord. One carotid sinus, say the left, of B retains its normal innervation, the other one is denervated. One suprarenal vein of dog B is anastomosed with an external jugular vein of dog C, which has been epinephrectomised, and its spleen is placed in a plethysmograph connected with a volume recorder. Any liberation of adrenaline from the suprarenal of dog B will cause a shrinkage of the spleen of dog C. This, together with a rise of blood pressure, happens when the left (normally innervated) carotid of B is clipped off, but not if the right (denervated) carotid is clipped off. The opposite happens on removing the clip from the innervated carotid, namely, a fall of blood pressure in B and expansion of the spleen of C. These effects vanish if the splanchnics of B are divided. This experiment shows that, as claimed by Tournade, rise of pressure in the arterial circulation of the head inhibits vasomotor tone and adrenaline secretion, while a fall of pressure has the opposite effects ; but further, and opposed to the original conclusion of Tournade, it shows that these effects are not due to direct action on the bulbar centres, but to a reflex arising from the carotid sinuses. A comparable reflex *viâ* the depressor nerve is also claimed by Richards and Wood and by Cannon and Rapport, who have found that the adrenaline output of the suprarenals is reduced on stimulation of the central end of the depressor nerve.

It should be noted that the participation of this mechanism in the depressor reflexes *viâ* carotid sinus or depressor nerve presupposes a constant output of adrenaline from the suprarenals. We cannot here take up a discussion of the question as to whether, under usual states of arterial pressure, there is a continuous discharge of adrenaline, which might be further reduced in response to a rise of arterial pressure, though Tournade and Chabrol, and many other physiologists are of the opinion that this is so ; the converse case, however,

that a considerable fall of arterial pressure sets in operation a reflex discharge of adrenaline, appears to the writer to be satisfactorily established.

Reciprocal Action in the Reflexes

In the reflex fall of blood pressure brought about by stimulation of the central end of the depressor nerve, it was shown by Bayliss that there was a reciprocal relationship between the effects on the vasoconstrictor and vasodilator centres. In the depressor response, the normal vasoconstrictor tone was inhibited, but, in addition, there was a positive vasodilator reflex. The latter was demonstrated, for example, when, on stimulation of the depressor nerve, there was an increased flow of blood through the submaxillary gland which had been deprived of its sympathetic vasoconstrictor impulses by section of the cervical sympathetic.

We should expect the reflexes generated from the carotid sinus when the blood pressure within it is increased to be similar in type to the depressor reflexes, and accordingly that the fall of systemic blood pressure would be due, not only to the inhibition of vasoconstrictor tone, but also to the setting up of a vasodilator tone. Conversely, when the pressure is lowered, not only would the reflex vasodilator tone be reduced, but the normal vasoconstrictor tone would also be re-established.

Certain of Heymans' experiments bear out this similarity and show that the reflexes in response to fall of arterial pressure are not due solely to release phenomena, *i.e.*, to inhibition or withdrawal of vagus or vasodepressor tonus, but are also accompanied by positive pressor and accelerating reflexes. Thus if in a dog with divided vagus and depressors both carotids are clamped, the fall in arterial pressure in the head causes a rise of systemic pressure and an acceleration of the heart. If these alterations are due solely to release

phenomena, then the condition should remain the same on denervation of the sinuses. Actually, on the contrary, denervation of the sinuses causes the blood pressure and the heart rate to fall towards the normal.

It would appear probable that, when the pressure is abnormally high in the carotid sinuses, a depressor reflex results, *i.e.*, vasomotor tone is reduced and the heart is slowed ; when the pressure is abnormally low, on the other hand, not only does this reflex cease to operate, but a counteracting pressor reflex is established, viz., increase of vasomotor tone and cardiac acceleration. A parallel case in the action of the vago-depressor was observed by Pavlov in 1879, and has been confirmed by McDowall : after very severe hæmorrhage, section of the vagus (plus depressors) instead of causing the expected rise of blood pressure, often causes a fall. The experiments of McDowall indicate that this is due to the abolition, by the section, of a pressor reflex. Anrep and Segall, from experiments on the innervated heart-lung preparation, suggest that the depressor reflex from the aorta is definitely put into action at pressures above 120 mm. Hg ; between 120 mm. and 80 mm. there was no depressor response, while below 80 mm. there was a pressor response in the head vessels, in which the depressor mechanism could not be involved.

We may thus consider that there are two important means of adjustment by which the body is protected from the effects of abnormal changes in arterial blood pressure. The carotid sinuses serve to regulate the arterial pressure so far as the vessels to the head are concerned, thus protecting the nerve centres ; the aorta, and perhaps also the ventricles with their depressor supply, are regulators of the cardiac and aortic pressures. That the arteries of the body generally have a subordinate innervation with analogous general functions cannot be admitted, though traces of reflexes of similar type certainly persist even after division of both depressors and denervation of both the carotid sinuses.

According to Heymans these subsidiary zones are located in the aorta, and do not extend beyond its bifurcation into the iliac arteries.

Participation of the Regulating Reflexes in Familiar Phenomena

An important part is played by these reflexes in enabling the body to counteract the effects of severe hæmorrhage. This, by causing a fall of general arterial pressure, would set in action the pressor aspects of both the "depressor" and carotid sinus reflexes, thus causing vasoconstriction and cardio-acceleration, so that the fall of pressure would be much less than it would be without this regulating mechanism.

Another aspect is that which concerns the effect of posture on the circulation. It is well known that when we rise from the recumbent to the erect posture there is a compensatory vasoconstriction and cardio-acceleration, the effect of which is to prevent the arterial pressure from suddenly falling, especially in the head vessels. On standing on the head, we can suppose that the reverse happens. These phenomena must play a large part in regulating the arterial pressure in the cerebral vessels of a long-necked animal, such as a giraffe, in which the fluctuation in hydrostatic pressure, if not so corrected, would be of the order of at least 150 mm. Hg, when the head was moved from the ground to its highest position.

These regulating reflexes provide yet another example, if any be needed, of one of the most fundamental of biological laws, namely, the law of the maintenance of constant conditions in the external environment of the tissues, whereby a small change in the conditions gives rise to reactions which oppose further change, and oppose it with a force which increases as the change to be opposed augments.

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CHAPTER III

THE CAPILLARY CIRCULATION

Access of blood to the capillaries is facilitated by dilatation and restricted by constriction of the muscular coats of the arterioles, while exit of blood from the capillaries is aided by dilatation and hindered by constriction of the venules which conduct it away. By local regulation of these controlling mechanisms much can be done towards ensuring a sufficient local supply of blood to the tissues. Of the control of the arterioles we have ample evidence. Of the separate activity of the venous vessels we have less information, but such as we have indicates that regulation of venous return by alteration in the calibre of the smaller veins is of far less importance than the control of other parts of the vascular circuit. But very often, particularly in the skin, the smaller venules react as capillaries, which indeed they also resemble closely, both in their minute structure and in their physiological functions.

It is now certain that the capillaries and small venules must be recognised as being subject to active alterations in capacity and resistance according to the wants of the tissues whose needs they serve.

It must be borne in mind, however, that the evidence that the capillaries are capable of active and independent contraction in no way diminishes the certainty of former conclusions regarding the control of the arterioles by similar means.

The Contractility of the Capillaries

Variation in the calibre of capillaries was observed under the microscope more than sixty years ago, but the evidence

available was not then such as to enable the conclusion of independent contractility to be established beyond question. The chief reason for this was that it is by no means easy to distinguish active changes in capillary diameter from passive ones. If an arteriole dilates, the capillaries which it supplies will receive blood at a greater pressure, and this will distend them passively ; the converse will happen when the arteriole constricts. It is difficult, if not impossible, to say in such an instance whether we are dealing with simultaneous active dilatation of both arterioles and capillaries or with an active dilatation of the arterioles alone with passive distension of capillaries ; and the same difficulty presents itself in the interpretation of constrictions. But a clear case could be considered to exist if it could be shown (*a*) that of the capillaries supplied by a given arteriole some could be caused to constrict while at the same time others did not, or (*b*) that the arterioles constricted while the capillaries dilated, or *vice versâ*. These and other proofs have now been abundantly supplied by several independent lines of study, some of which will now be briefly described.

1. Observation with the Microscope

Contraction of capillaries can be actually seen to occur while the circulation is watched under the microscope, *e.g.*, in the web of the frog's foot, the nictitating membrane of the frog, the frog's tongue, the omentum and muscles of frog and mammal, the glomeruli of the frog's kidney, the pancreas of the rabbit, the human skin, the tail of the tadpole, etc. The first clear demonstration of active capillary contraction seems to have been given in 1879 by Roy and J. Graham Brown. They found, on applying graduated pressure to a transparent tissue in which the circulation was under observation, that some of the capillaries closed before others, but that on repeating the application of pressure it was not always the same

capillaries which closed down first. It was concluded from this that the tone of the capillary walls was different from time to time. They also observed that local asphyxia, produced by temporary stoppage of the circulation, caused dilatation of the capillaries when the blood was readmitted.

Simple Methods.—Contractility of the capillaries may be

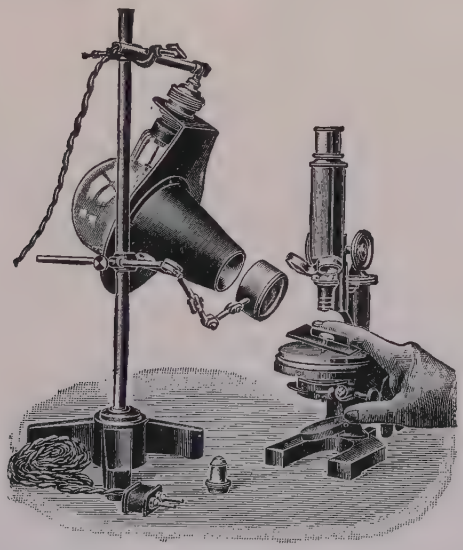

FIG. 22.—Arrangement of illumination for viewing skin capillaries (Professor Eugen Weiss). (From Abderhalden's "Handbuch der Biologischen Arbeitsmethoden," by permission of Messrs. Urban and Schwarzenberg.)

demonstrated on almost any tissue in which these vessels can be observed during life. Surface capillaries in the human skin are observed by throwing a strong beam of light filtered through a blue screen obliquely on to the surface of the skin at the desired spot (Fig. 22), which is covered with a layer of thick cedar oil or liquid paraffin. A better view is obtained if the horny layers of the epidermis have been removed, as by peeling off after production of a blister.

If the skin of the back of the hand or that at the root of the nail is examined in this way, preferably with a binocular microscope, capillary loops can be seen.

Krogh has shown that the frog's tongue is also a very good object on which to study the reactions of the capillaries. If the mucous membrane on the under-surface of the tongue be pinned out for examination as a transparent object, many new capillaries are at first opened up, and a rich supply of blood results ; but on allowing the preparation to stand in a moist atmosphere these capillaries vanish from view again, and the tongue once more becomes pale. If now a very light scratch be given to the upper surface at any point by means of a hair, one or more capillary vessels will be seen to open up beneath the stimulus, and ultimately to establish connection with a small vein. Such simple observations as these show that the capillaries are independently contractile, and that they may sometimes contract down to such an extent as to prohibit any flow of blood through them.

The observation of alterations in size of the capillaries is greatly facilitated by the introduction of graphite ink into the circulating fluid as recommended by Drinker.

Injection Method.—Further evidence for capillary contractility has been furnished by Krogh by the method which he calls " vital injection," which shows that many capillaries are so firmly contracted as entirely to resist the passage of any fluid. India ink is dialysed against Ringer's solution, and is then injected into the circulation, the animal being afterwards killed and the tissues, after suitable fixation, examined under the microscope. The capillaries which were open will contain blood corpuscles and ink, and will stand out clearly, while those that were quite closed will be invisible under the microscope. Comparison by this method of the numbers of capillaries in resting with those in stimulated muscle shows a very great increase in the number of capillaries which are patent when the muscle is active. Thus, in the frog the resting sartorius showed in one experiment 85 to

100 capillaries per square millimetre of cross-section, while after stimulation the number was 305 to 340 per square millimetre. In the brain, on the other hand, all the capillaries appeared to be open at all times. In mammalian tissues, as may be expected, the capillaries during activity are much closer together than in those of the frog; thus, in the diaphragm muscle, which may be regarded as always active, Krogh counted as many as 2,700 capillaries per square millimetre of cross-section, the average distance between two adjacent capillaries being only 18 μ.

In contracting muscles not only are more capillaries open, but the average diameter of the capillaries which are open is greater than in the resting muscle. Thus Krogh found the following diameters (twenty capillaries measured in each case):—

	Frog (*Sartorius*).		Guinea-pig.	
	Rest.	Stimulated.	Abdomen (rest).	Diaphragm (active).
Maximum .	10·6 μ	10·7 μ	6·5 μ	10·4 μ
Minimum .	2·1 μ	4·1 μ	1·8 μ	2·2 μ
Average .	4·3 μ	6·8 μ	3·5 μ	5·0 μ

Since the red corpuscles in the frog measure about 22 μ in length by 15 μ in breadth, with a maximum thickness of 4 μ, while those of the guinea-pig are 7·2 μ in diameter and 2 μ thick, it is evident that even when the capillaries are dilated not all the corpuscles can pass through all of them without deformation, while when they are only partially open the corpuscles must all suffer great change of shape in filing through any of them. This is illustrated by Fig. 23, which shows four capillaries from the abdominal wall of the guinea-pig. When the diameter of the capillary is about 4 to 5 μ, the corpuscles are rolled up in passing, as shown

by No. 5, while in still narrower vessels, as in No. 3, the corpuscle may in squeezing through assume a sausage shape 14 μ or more in length.

A modified injection method has been employed also by Rous and Gilding for the study of the relative circulatory service to different parts of the body. Their method is to

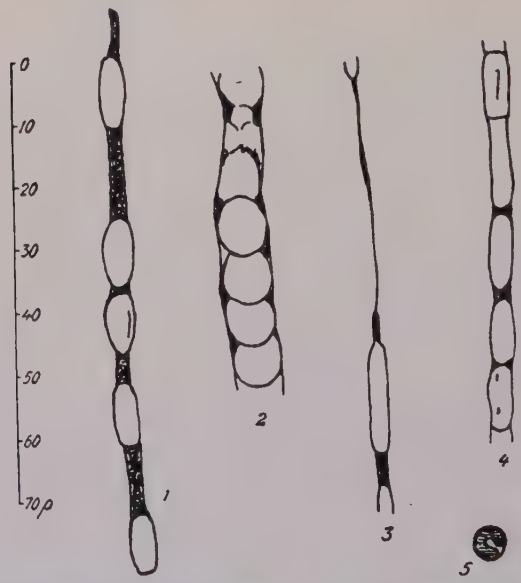

FIG. 23.—Different state of contraction in four capillaries in muscle of guinea-pig. (Krogh.)

observe the speed and depth of staining of the tissues after injection into the blood stream of highly diffusible dyes, such as Patent Blue V., or brom-phenol blue. Their results show that, even after a severe hæmorrhage, the essential internal organs are very richly supplied with blood, as compared with the muscles and skin. The diaphragm is, however, always well served. Gilding obtained similar results by the use of India ink injections.

Contractile Structures of the Capillaries.—When contractility of the capillaries had been definitely demonstrated, it became necessary to inquire into the means by which this was accomplished.

It is claimed by Vimtrup and by Krogh that the contractile structures associated with alteration of the lumen of the capillaries are branched cells placed outside their walls, very transparent and only demonstrable with some difficulty. Such cells were originally described by Rouget in 1873, and are called the *Rouget cells*.

This function of the Rouget cells has, however, recently been denied by Florey and Carleton, who claim that the motor activities reside in the endothelial elements of the capillaries.

Clark and Clark hold similar views, and have indeed shown that in the capillaries of the tadpole's tail contractility appears before the Rouget cells are developed. It seems probable, therefore, that Aschoff's opinion that these cells are related to histiocytes is more correct.

2. Naked-eye Observations on Skin Vessels

A number of long familiar observations by the naked eye receive a more satisfactory explanation now that the contractility of the capillaries has been established by the microscope. The most interesting of these are the *skin reactions* which have been studied by Lewis, by Ebbecke, Weiss, and by Miss Carrier; some have correlated the naked eye phenomena with microscopic observations.

Such observations refer to the vessels to which the depth of colour of the skin is due, and these are mainly the minute venules of the subpapillary plexus; these show the same reactions as the capillaries to which, actually, only a small part of the skin colour is due.

In carrying out experiments on the colour of the skin it is useful to have a series of standard colours with which comparison can be made. Such a series of standards has been described by Lewis (1929), and can easily be made.

If a blunt point is drawn over the skin, particularly in the interscapular region, a mark, or *tache*, is left behind. The nature of the *tache* depends on the intensity of the stimulus, *i.e.*, on the instrument and pressure employed, and on the responsiveness of the vessels in the skin. On gentle stroking, or on applying pressure with the finger the *tache* is white, and appears in fifteen to twenty seconds, being preceded by transient flushing. Both of these reactions, the red and the white, are due to changes in the calibre of the skin capillaries and small venules. When the point is drawn over the skin with a firmer pressure, there is first a light flush (capillary dilatation), followed in fifteen seconds or so by (*a*) a much deeper flush along the actual track of the instrument (wide dilatation of capillaries and possibly of other vessels also), and (*b*) at either side of the red *tache* so produced there appears a white *tache* (capillary constriction). These fade in from three to eight minutes. Microscopic inspection shows that the capillaries and venules in the pale area, though constricted, are in connection with vessels filled with blood, and the emptiness of these vessels can only be due to the fact that they have actively contracted against the pressure of blood with which they are supplied.

White reactions can also be produced by introducing adrenaline into the skin, *e.g.*, by pricking with a needle through a drop of the solution.

A measure of the strength of the contraction of the capillaries may be obtained by the following simple experiment, as shown by Sir Thomas Lewis (congestion test). A subject who exhibits the white *tache* well is chosen, and a Riva-Rocci armlet placed on the upper arm. The white *tache* is produced by stroking the skin of the forearm, or by adrenaline, and when fully developed, the armlet is steadily pumped up, when it will be found that at a certain pressure the white *tache* suddenly disappears. When this happens, the pressure registered in the armlet is equal to the venous pressure which has been produced by its congestion, and the

capillary pressure cannot be less than this. It is usually found that the white line does not vanish until a pressure of about 50 to 100 mm. Hg has been reached ; a pressure at least as great as this is hence required to force open these contracted capillaries, which must, therefore, be able to contract with considerable strength.

Another simple experiment is Lewis's *Occlusion test.* A Riva-Rocci armlet is placed in position and *rapidly* inflated to a pressure greater than the arterial pressure, *e.g.*, 200 mm. Hg. The circulation in the arm is thus suddenly stopped, and the pressure in arteries, capillaries, and veins then becomes equal ; as the inflation was rapid, this pressure, though higher than the usual capillary pressure, is not very great. On now applying the skin reaction test the white *tache* will be found to develop in the usual manner. Now, since the colour of the skin is due to blood contained in the minute vessels, it is evident that in such a limb, with occluded vessels, a contraction of arterioles could not deprive the capillaries of their contained blood, as might be objected if the circulation were proceeding.

Lewis has shown that the red reaction consists of two distinct reactions—a localised one due to relaxation of all the skin vessels, and a spreading flare due to relaxation of the strong arterioles often for considerable distances, when powerful stimuli have been used.

In a certain number of subjects the skin after the *tache* tests shows some urticaria, while in others a definite wheal results owing to the production of local œdema—a condition called *dermatographism.* The *triple response*, local vasodilatation, spreading flare and local œdema may be obtained by stimulation of the skin by mechanical means, heat, cold, electricity and various "irritant" substances, especially histamine.

Certain internal organs also show reactions when their surfaces are stroked ; thus the liver most readily shows the red reaction, and the kidney the white one.

As a generalisation which is found often to be of value in clinical work, we may say that the *colour* of the skin is determined by the number and degree of filling of, and rate of circulation in, its minute vessels, while the *temperature* of the skin depends upon the rate of circulation through its arterioles. Thus we may have a cold pale hand in which both types of vessels are constricted, a warm pale one in which the arterioles are dilated, but the capillaries only moderately so, a warm red member in which both are dilated, a warm blue one in which the capillaries and venules are dilated but the circulation insufficient to prevent cyanosis, or a cold red one in which arterioles are constricted, but capillaries dilated, as when the hands are exposed to extreme cold.

An important factor is, of course, the rate of reduction of the blood. An interesting experiment in this connection is described by Lewis. One hand is placed in ice-water, the other in hot water; on withdrawing after several minutes both appear bright red. Now the circulation is stopped in both arms by sphygmomanometer armlets—almost at once the hand from the hot water turns black, but the other one becomes cyanosed only very slowly.

3. Observations by Methods of Experimental Physiology

Certain substances, when injected into the blood stream, produce extreme dilatation of the capillaries. The most striking result is a very great fall of arterial blood pressure with enormously distended capillaries, which often show hæmorrhages.

The best documented study of the action of a capillary poison is that of Dale and his co-workers on *histamine*. The earlier experiments by Dale and Laidlaw showed that when injections of histamine were given to carnivora there was a fall of arterial pressure. Dale and Laidlaw further showed that when histamine in large doses (1 to 2 mg.) was administered intravenously to an etherised cat there was a large and lasting fall of arterial blood pressure, and although the

heart was still beating vigorously the arteries and veins were almost empty. The use of smaller amounts of histamine (0·01 to 0·1 mg.) gave a transitory effect, which was fully studied by Dale and Richards. Tracings of the limb volume showed that there was conspicuous dilatation, and it might be thought that this dilatation was due to relaxation of the arterioles. But when a perfusion experiment was carried out, it was found that histamine led to a diminution of flow through the tissues, which suggested that the arterioles were not relaxed, but actively contracted, by the drug. This opinion was supported by the further fact that the action of histamine on portions of isolated artery was to cause constriction, just as it did on most other varieties of plain muscle. The substance *acetyl choline* provided an excellent contrast: when this was injected into the etherised animal, a fall of blood pressure, with dilatation of the limb, was also produced; but in this instance the fall was due to relaxation of the arterioles, for when a limb was perfused with blood the addition of 0·001 mg. of acetyl choline caused moderate dilatation, with a very large increase of blood flow.

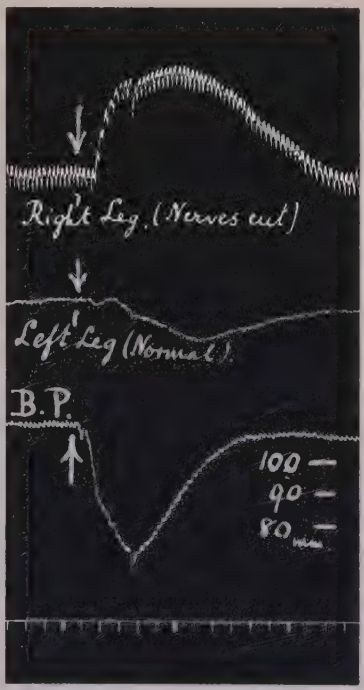

FIG. 24.—Effect of 0·01 mg. histamine on arterial pressure and limb volumes, right leg freshly denervated, left leg normal. (Dale and Richards.)

After recent section and even after degeneration of all the nerves to a limb Dale and Richards found that the dilator effect of histamine was not abolished, but usually much intensified (Fig. 24). Soon after division of the nerves there was evidence that the arterioles of the limb were in a permanently dilated state, while the capillaries were tonically constricted. Thus the pulsations on the limb volume tracing were greatly

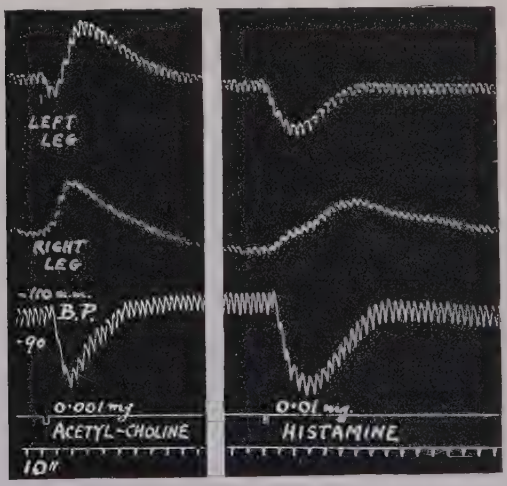

FIG. 25.—Effect of histamine and acetyl choline on arterial pressure and limb volumes; left leg completely denervated three weeks previously, right leg deprived of sympathetic innervation twenty weeks previously. (J. H. Burn.)

exaggerated as compared with the normal limb (Fig. 24), while the pad of the foot was warm, though pale. In the normal limb, the volume pulse was small, and the pads red, but cold. When a dose of histamine (say, about 0·01 mg.) was given intravenously, the two limbs exhibited a pronounced contrast in behaviour, the pale warm denervated limb showing with good expansion a flushing of the pads, while the normal limb showed a smaller expansion, or even a passive shrinkage, owing to the fall of blood pressure (Fig. 24).

The reason for this difference is to be found in the very different degree of capillary tonus before administration of the histamine. The denervated limb had relaxed arterioles, but tonic capillaries, while the normal limb had tonic arterioles and dilated capillaries. It seemed, therefore, that there was some factor other than the nerve supply which was responsible for the maintenance of capillary tonus. At the same time, further experiments by Burn have shown that after section of *all* the nerves to the limb, though not after sympathetic denervation alone, the dilator response to

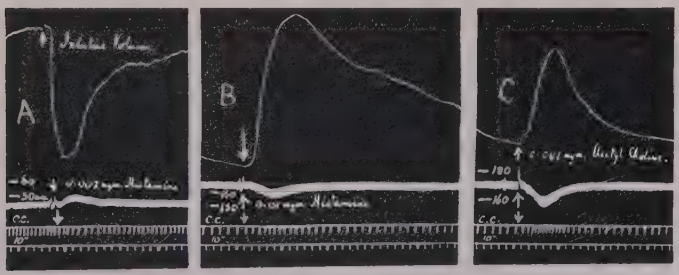

FIG. 26.—Perfusion of intestine with blood. Upper tracing = organ volume; second tracing = perfusion pressure; third tracing = blood flow through tissue; lowest tracing = time. A = before, B and C after, adding 1 part adrenaline per million of blood. (Dale and Richards.)

histamine is usually lost when complete degeneration of the divided nerves has taken place (Fig. 25). Burn suggests that the other factor, in the absence of which the capillary tone is ultimately lost, may be the integrity of the posterior root fibres, and raises the interesting question whether the trophic changes which are known to follow sensory denervation may not be due to the altered conditions of capillary circulation.

A fact which suggests the participation of certain chemical conditions in supporting capillary tonus was discovered by Dale and Richards, who showed that normal responses could be obtained even with perfused organs or limbs provided

that the blood was well oxygenated and contained minute amounts of adrenaline (*e.g.*, 1 in 10 million) (Fig. 26). Under these circumstances histamine produced dilatation of the limb volume, together with moderate increase of blood flow. The explanation was that under these conditions there was a state of moderate tonus of the capillaries, so that the dilator action of histamine could be revealed.

It may be mentioned here that adrenaline in small doses also produces a transitory fall of blood pressure, which Dale and Richards explained as being due to a dilator action on the capillaries similar to that of histamine, since the two actions run parallel. In larger doses, of course, adrenaline causes constriction of the arterioles, and also, it is believed, of the capillaries.

The Reactions of Capillaries to Stimuli

Innervation of the Capillaries.—Histological investigations have revealed the presence of non-medullated fibres, believed to be of sympathetic origin, as a terminal nerve net of primitive type around the smaller vessels. On stimulation of the sympathetic supply, contraction of the capillaries, as well as of the arterioles, has been observed.

It seems equally certain that the capillaries have a vaso-dilator supply closely resembling that of the arterioles, which was discovered by Bayliss and called the antidromic supply. Stimulation of the posterior nerve roots has been shown by Doi to cause dilatation of the capillaries even after the arterioles have been fully dilated by the injection of acetyl choline. It appears from these and other similar experiments that the vaso-dilator antidromic responses of the vessels involve both arterioles and capillaries, and that similar conditions apply to the reactions of both. Thus in herpes zoster, in which local areas of skin flushing are associated with irritative lesions of the posterior roots, the dilatation involves vessels of both types. Again, it has

been shown by Bruce and by Breslauer that the hyperæmia produced in the conjunctiva and in the skin at the site of application of mustard oil depends on the integrity of the nerve supply, and is probably an antidromic response of the axon reflex type, since it persists for some time after nerve

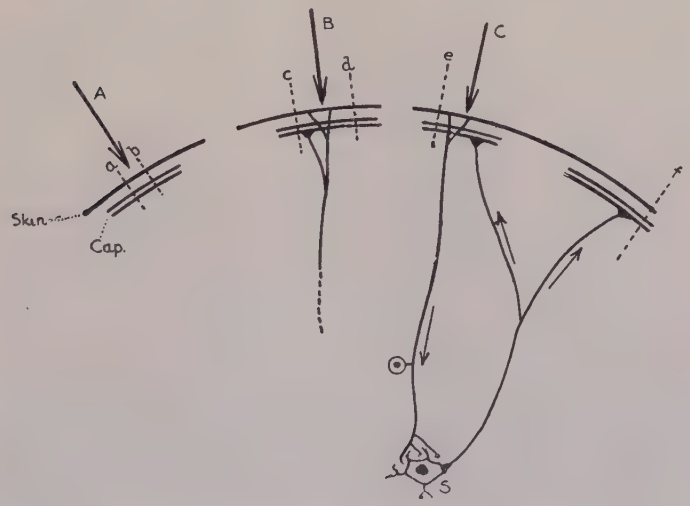

FIG. 27.—Diagram to illustrate the three possible types of response of skin vessels to stimuli applied at A, B, and C. A, direct effect on capillaries, affecting area *a*—*b*. B, axon reflex on arterioles (and capillaries ?), affects *c*—*d*. C, possible, though improbable, spinal reflex, widely spreading over area *e*—*f*.

section, but disappears after degeneration of all the nerves, or after the induction of local anæsthesia by novocaine. These results have also been confirmed by Lewis, who, however, points out that this spreading flare in which the axon reflex is involved is due principally to dilatation of arterioles; it disappears about six days after section of the nerve supply.

In addition to these purely local effects, we have to take into account the possibility of the occurrence of true reflexes in which the spinal cord is involved. So far as the skin is

concerned, Lewis considers that the evidence is not sufficient to justify the belief that they represent a definite or important mechanism.

To summarise, we may say that there are two ways in which the skin, at all events, may show a dilator response to a localised stimulus: first, a direct effect of the mechanical stimulus on the capillaries; secondly, an axon reflex: a widely spreading response due to an actual spinal reflex seems unlikely. These types of response are illustrated diagrammatically in Fig. 27.

Reaction to Light.—The capillaries of the skin are sensitive to the action of ultra-violet and violet rays, and the erythema of sunburn is due to this cause. The result is a dilatation, which comes on after a latent period of a few hours, lasts for several days, and leaves the capillaries in an excitable state to mechanical stimuli for some months, though less sensitive to a second application of light.

Effect of Temperature.—Moderate cold dilates and warmth constricts the skin vessels when applied locally; extremes cause damage, and hence may give triple responses. Whitening of the skin by cold is regarded by Lewis as abnormal.

Capillary Pressure

The pressure in the capillaries is low but variable. It is raised when there is a dilatation of the arterioles, and lowered when these constrict. Experiments by Leonard Hill have shown that the capillary pressure is not often so high as has been previously supposed, and in the tissues of the frog, toad and newt lies between 0·5 and 6 cm. water; similar values were obtained for the ear and mesentery of mammals.

Capillary pressures have also been measured in man by Carrier and Rehberg as follows: A fine glass capillary is filled at the point with saline solution and pushed into a capillary which is under observation under the microscope. Attached to the other end of the capillary is a water

manometer, by means of which a definite pressure is applied to the column of saline solution in the capillary glass tube. When the blood capillary is pierced, blood will flow into the glass capillary if the capillary pressure is higher than that in the water manometer; if lower, no blood will enter; if the same, blood will pulsate in and out of the glass capillary at each heart-beat.

In this way it was found that the capillary pressure in the hand varies with the position of the hand with reference to the heart, and may be as great as 30 cm. water, or as little as 4 or 5 cm.; but, however high up the hand was placed, it never fell below a certain level, of about 4 to 5 cm. The reason for this is because the veins collapse down and restrict the flow when the hand is held in such a position that the venous pressure becomes negative. The capillary pressure is thus always maintained at a positive value, which varies according to the position. In the feet the capillary pressure is normally prevented from attaining very high values owing to the presence of valves in even the smallest veins, and to the occurrence of constant small muscular movements, which, by forcing the blood into these veins, enables them to act as a kind of pump.

The Permeability of Capillaries

The walls of the capillaries are thin and well adapted to allow ready diffusion of substances through into the tissues. Water and crystalloid substances pass through readily, but the proteins and other colloids of the blood are held back more or less completely. The size of colloid particles in blood is very variable, however, and Krogh and his collaborators have devised a method for studying what they call the fractional osmotic pressure of the blood, which enables an estimation to be made of the relative proportion of colloid particles of large or small size. This depends on the fact that when blood is dialysed against Ringer's fluid an osmotic

pressure is established which depends on the concentration of those substances present in the blood which are unable to pass through the pores of the dialysing membrane. Provided it be permeable to water, the less permeable the membrane the greater will be the osmotic pressure set up by its contents.

The membranes which were prepared were permeable to water and salts, but varied in their permeability to colloids, which were thus strained off, as it were, according to the size of their particles. Using dialysers of increasing permeability, which may be called Nos. 1, 2, 3 and 4, the following average results were obtained :—

Blood.	Temperature.	Osmotic Pressure in mm. Water in Dialysers.				Average Osmotic Pressure per 1 per cent. of Protein in Blood, mm. H_2O (calculated from No. 3).
		1.	2.	3.	4.	
Frog . .	15°	125	55	57	29	33
Rabbit . .	37°	333	295	275	247	53
Human . .	37°	710	477	457	<70	61

The maximum osmotic pressure which is obtained in each case shows a rough proportionality to the protein content of the plasma, which is about 2 per cent. for frog's, 5 per cent. for rabbit's, and 8 per cent. for human blood. It may be assumed that the particles which are allowed to pass through by the No. 3 dialyser and by the lower numbers are colloids other than proteins, while No. 4 allows even a part of the proteins to pass out. But in rabbit's blood the part passing out is much smaller than in human or frog's blood.

It is quite reasonable to suppose that, if the permeability of the capillaries were to be increased, some of the smaller-sized protein particles might pass through their walls, and this seems certainly to be so. Starling has shown, for instance, that in certain parts of the body the osmotic pressure of the serum proteins is, as in the most permeable of Krogh's dialysers,

so low that it falls below the normal capillary pressure, and lymph filters through with a protein content almost equal to that of the plasma. This happens under normal circumstances in the liver and in the intestine. The most important fact in connection with capillary permeability is that it is increased under circumstances which lead to active dilatation of the capillary vessels. When a dose of histamine was given to a cat under ether, for instance, it was found by Dale and Laidlaw (1919) that there was a rapid concentration of the blood cells, which was ascribed to an increased rate of capillary filtration. In one case the red cell count of the blood rose to the high figure of $13\frac{1}{4}$ millions per cubic millimetre, while the hæmoglobinometer and hæmatocrite readings also showed high concentration of the blood in histamine shock, as seen in the following table :—

Experiment.	Hæmoglobinometer.			Hæmatocrite.		
	Normal.	Shock.	Ratio.	Normal.	Shock.	Ratio.
1	78	116	1·49	33	50	1·5
2	80	120	1·5	33	50	1·5
3	92	130	1·4	33	50	1·5
4	80	118	1·3	40	52	1·3

The same observers were also able to show that there was a considerable increase in the flow of lymph from the thoracic duct after administration of histamine, and further, by the use of the vital red method, that there was a diminution of blood volume. Under these circumstances the capillaries may be regarded as so damaged that they become abnormally permeable. Dale and Richards found, for instance, that the application of histamine to the surface of the pancreas led to intense congestion and the development of definite œdema. Abel and Geiling have found an alcohol soluble fraction of peptones which had all the effects of histamine. It seems extremely likely that the lymphagogue

action of peptones is due to the presence in them of histamine, or substances closely resembling it.

These facts are of great importance in connection with the normal function of the capillaries in supplying to the tissues the materials which they require during increased activity, in which, of course, the capillaries are in a dilated state. After the capillaries have returned to the normal condition again the permeability is also restored to normal values. In no case is there evidence that the normal capillaries ever become permeable to visible particles. Krogh found, for instance, that particles of Indian ink which were 200 $\mu\mu$ in diameter, and beyond the limits of visibility, were unable to pass through the walls of even widely dilated capillaries. Soluble starch, however, which had particles of 5 $\mu\mu$ in diameter, was kept back by contracted or tonic capillaries, but passed through them when dilatation was produced, and the same applied to certain colloidal dyestuffs.

When the capillaries are very widely dilated and highly permeable, and particularly if the capillary pressure at the time is not low, the outward passage of fluid from the vessels may be so rapid that the concentration of the red cells may be watched under the microscope and seen to culminate in the formation of a practically solid mass of corpuscles, blocking up the capillary and producing stasis. This state of affairs may be seen, for instance, in Krogh's experiment, in which a small drop of urethane is applied to the surface of the frog's tongue.

These facts are of importance in connection with the production of œdema. There is normally a relation between the resistance to filtration through the capillaries in any region and the capillary pressure which prevails there, and if the former is lowered the filtration may occur at such a rate as to lead to the development of œdema. The œdema fluid of wheals contains almost as much protein as plasma. The whealing of the skin which often follows mechanical, chemical or other stimulation has been studied by Lewis and Grant,

who find a close relation between flare production and wheal formation. The formation of a wheal is not accelerated by venous congestion nor by application of suction to the skin, though these accentuate the filtration pressure. On the other hand, it requires a positive pressure of about 40 mm. Hg applied to the skin to prevent wheal formation. Evidently, therefore, altered permeability of the vessel walls is the principal factor, provided, of course, that the rate of blood flow is adequate. Mere stretching of the vessel walls does not lead to œdema.

Under some conditions venous congestion is probably associated with altered permeability, so that it appears to be a causative factor in producing œdema. This is well seen in the legs and feet when for any reason the capillary pressure is raised, as in certain heart affections or in pregnancy. If the subject stands about and the vessels become congested, œdema follows ; it is relieved by rest in a horizontal position. Blistering appears to be essentially similar to whealing, but appears more slowly.

When a wheal has been produced by any of the usual methods, and is subsiding again, it is found that a second application of a whealing stimulus, or a local injection of histamine, does not lead to whealing. This refractory condition is believed by Lewis to be due to some change in the vessels and not to failure of the tissues to liberate the H-substance.

Reactive Hyperæmia.—If the circulation in a limb or area of skin is stopped for some minutes, *e.g.*, by the application of a tight bandage, and then restored, there is an immediate dilatation of the minute vessels and an accelerated rate of circulation in the skin, which lasts for about half as long as the arrest. This response is seen equally well in denervated vessels and appears to be due to the action on the minute vessels of metabolites, such as the H-substance, which have accumulated in the tissue spaces during interruption of the blood flow. The response may be followed by œdema

if the interruption has been very long, but under normal conditions represents an important means of regulating the blood supply in accordance with the needs of the tissues.

When stoppage or reduction of the circulation has been of longer duration, it was found by Gilding and Rous, that, after release, patches of persistent constriction occur in the skin vessels. These they believe to be related to what are known as Bier's spots—similar ischæmic patches of skin which stand out during a period of occlusion of the circulation in a limb. Since they find that Bier's spots can prevail over the local vaso-dilatation due to injury to the skin, causing this to blanch, but cannot similarly overcome the dilatation caused by pricking-in of histamine, they are inclined to doubt the validity of the hypothesis which refers all local vaso-dilatations to the action of a histamine-like substance.

The Causation of Capillary Tone.—Krogh is of the opinion that a pituitary hormone is the chemical factor which is chiefly responsible for the maintenance of capillary tonus, while Dale and Richards think that it is possibly adrenaline. There is certainly some substance present in blood serum which Krogh has shown is dialysable, though the evidence that it is either of the substances suggested above is only presumptive.

When some of the capillaries in certain tissues have been closed, it has been shown that after a time they open up, and other previously patent capillaries close down in their place. The mechanism by which this alternate opening and closure of the capillaries is effected is not quite clear. It may be that the hormone responsible for the upkeep of the capillary tonus gradually disappears, thus leading to relaxation, or it may be that metabolites produced in the tissues around the occluded vessel lead to its dilatation.

Krogh's evidence that it is a pituitary principle which is responsible for the maintenance of capillary tone is, briefly, as follows: (1) Addition of pituitrin to the circulating blood does actually increase the tonus of the capillaries. Recent attempts by Drinker to repeat this observation were not successful, but it is easy to show by puncturing dilute

pituitrin into the skin that an area of pallor results, however this may be interpreted. (2) In the frog, pituitrin also acts on the melanophores of the skin, causing their expansion, so that the skin looks darker in colour. (3) It is possible to dialyse out from the blood a substance which raises the capillary tonus. (4) The dialysate so prepared also causes expansion of the melanophores, and is, therefore, presumed to be pituitrin. (5) After removal of the pituitary body from the frog the capillaries show a loss of tone, while the melanophores are contracted, so that the skin looks pale in colour.

It was shown by Lewis that intravenous injection of pituitary extract produces intense pallor of the skin, with only trifling rise of the blood pressure. Further, the injection will restore the blood pressure in a cat which has been brought into a state of profound vascular shock by the administration of histamine.

There is distinct evidence by Lewis and Grant, however, that reactive hyperæmia is in no way connected with disappearance of the pituitary principle from the blood or tissues. One of their experiments is easily repeated ; dilute (1 : 100) pituitary extract is pricked into the skin and the effect of subsequent reactive hyperæmia is observed. It is seen that the pale areas flush during the hyperæmic response and then blanch again as this dies down ; the response thus takes place in presence of excess of pituitary principle, and the latter is not thereby destroyed or removed.

Dale, on the other hand, has produced evidence in favour of the view that it is adrenaline which is normally responsible for the upkeep of capillary tone. Thus, in addition to the perfusion experiments quoted on p. 61, he has shown that after removal of the suprarenals from a cat the animal becomes exceedingly sensitive to the action of histamine, which will now cause death from histamine shock even in doses of 0·5 mg. per kilogram. Kellaway and Cowell have contributed further evidence in support of the suggestion

that histamine and adrenaline are physiological antagonists, for they have demonstrated that when histamine is injected into the cat there is an increased output of adrenaline from its suprarenals. When histamine is given, we have seen that there is, as a contributory factor to the causation of histamine shock, a concentration of the blood owing to loss of plasma. Such an oligæmia is seen after removal of the suprarenals, and is much intensified in these animals by the administration of histamine. The administration of adrenaline is shown to be capable of holding this blood concentration in abeyance both after removal of the suprarenals and after the administration of histamine. It would seem, then, that the administration of histamine or the removal of the suprarenals relaxes the tone of the capillaries, and so accelerates the rate at which plasma is lost through them by filtration; while the administration of adrenaline, either artificially or from the animal's own suprarenals, restores the tone and prevents the loss of fluid from the capillaries.

New Aspects of Hæmodynamics Introduced

The Peripheral Resistance.—As a result of these discoveries concerning the capillary circulation, it becomes necessary for us to modify somewhat our previous conception of the site of the peripheral resistance in the circulation. It has in the past been tacitly assumed that practically all the peripheral resistance is located in the arterioles, because it was believed that at these parts of the circuit, in spite of their rather large total sectional area, the individual vessels were, or could by constriction become, small enough to exert a considerable frictional effect on the relatively swiftly moving blood stream. In the capillaries, on the other hand, the total bed was so wide, and the movement of the blood therefore so slow, that, in spite of the small size of the individual vessels, their frictional effect was considered to be insignificant. And when the capillaries are all maximally dilated there seems to be no reason to doubt the correctness of this line of

reasoning. The slope of the pressure curve in different parts of the circulation would then be similar to that obtained after a dose of histamine, as shown by the curve EF in Fig. 28.

Under normal conditions we may now believe that of the total capillaries some are widely dilated, many are fully constricted, and some display moderate tonus. Owing to the

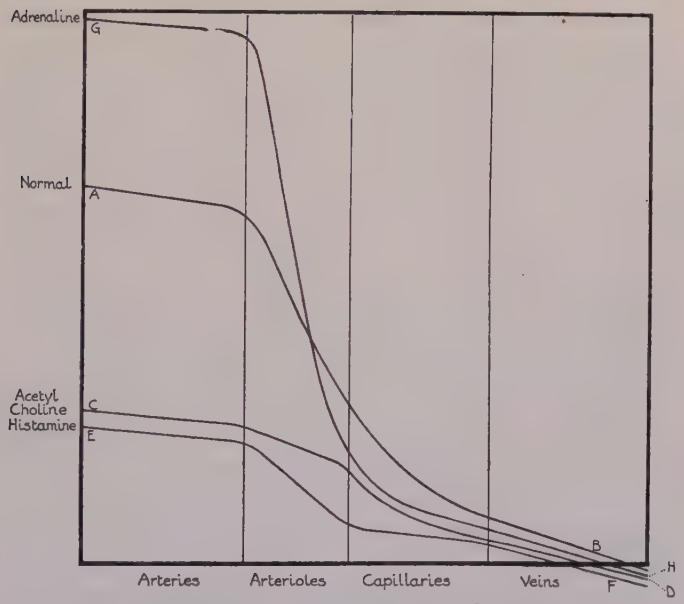

FIG. 28.—Pressure gradient in different parts of circulation, varying with condition of arterioles and capillaries.

narrowing of the bed, the velocity of the blood in these capillaries is now much greater, and the narrowness of the vessels makes it inevitable that a considerable amount of frictional resistance is offered to the blood stream; the pressure slope now shows a decided gradient in the capillaries as well as in the arterioles, as illustrated by the curve AB of Fig. 28. Dilatation of arterioles alone, as after the addition of acetyl choline to the blood, would give a pressure slope of

the type shown by the curve CD. When the arterioles and capillaries are both intensely constricted, as after the administration of adrenaline, then the distribution of pressure is as shown by the curve GH. We thus see that, although the arterial pressure may be altered by change of resistance in either the arterioles or capillaries, the type of pressure distribution will depend on which of these vessels is affected.

Secretion of Urine.—Another new aspect of modern views of the capillary circulation is in connection with urinary secretion. Richards and Schmidt have examined the glomeruli of the frog's kidney in the living state, and found that the glomeruli have periods of constricted closure alternating with periods during which they permit the passage of blood. This introduces an entirely new factor into the problem of urinary secretion, as it has previously been assumed that all the glomeruli were always open to circulation. It is quite conceivable that under certain circumstances all the glomeruli might be opened up, while at other times most of them would be closed, as no doubt would happen after adrenaline injections.

The Effect of Capacity.—*Surgical Shock.*—It is obvious if at any one time a very large proportion of the enormous total number of capillaries in the body may be shut down, while at another time they may be opened to the blood stream, that such change must produce alterations in the capacity of the vascular system which are incomparably greater than could be effected by any ordinary alterations in the tone of the arterioles. Krogh calculates, for instance, that the volume of blood in the active muscles of the guinea-pig may be 275 times as great as when at rest, while if all the capillaries were dilated fully there would be 750 times as much. Evidently, then, when extensive dilatation of the capillaries occurs, we have to take into account not only the reduced peripheral resistance, but also the greatly increased capacity of the vascular system. This is one of the factors which are responsible for the seeming disappearance of blood

from the circulation after the administration of large doses of histamine, the blood percolating into the lax network of channels as into a sponge. The heart, though beating as vigorously as ever, has but little blood to expel, and the animal "bleeds into its own dilated capillaries," so that heart and veins become emptied. In internal organs the capillaries may be seen to be flushed and distended with blood. In addition to this, there is, as already explained, an actual loss of fluid by percolation through the walls of the dilated capillaries, so that there is also a real loss as well as an apparent loss of fluid from the circulation.

Great practical importance attaches to these facts in connection with the pathology of surgical shock, or "secondary shock," and the shock-like state of collapse which is often seen in the terminal stages of general infections.

The central fact in surgical shock is a loss of blood volume, often in part due to hæmorrhage, but chiefly to (*a*) loss of plasma by filtration to tissues; (*b*) stagnation of blood in the peripheral parts of the circulation, especially the capillaries. In generalised infections there is a similar state of affairs.

The similarity between these conditions and the shock induced by large doses of histamine, as studied by Dale and Laidlaw, is so close as to suggest that they have not only similar manifestations, but a similar, if not a common, fundamental origin, to wit, that the clinical conditions are due to the action of histamine or substances resembling it in their action, and which are produced as a result of damage to the tissues. Though this hypothesis has not been directly proved, it has much to support it. Histamine can readily be extracted from nearly all tissues after death, and it seems not unreasonable to suppose that such substances, liberated from tissues damaged in the course of accidents or operations involving much handling, may find their way into the general circulation. There is direct experimental proof that this is so, for Cannon and Bayliss and others have shown that the crushing of a limb in an anæsthetised

animal results in a shock-like state, but if the vessels of the injured limb are occluded before the mechanical injury is effected, shock does not result until the vessels are released, so that the products of injury are admitted into the general circulation.

It is also of interest in this connection to note that hæmorrhage, anæsthetics, such as ether or chloroform (but not nitrous oxide (Dale)), or exposure to cold lower the resistance of the animal on the one hand to the action of histamine and on the other hand to the development of surgical shock.

Tissue Trauma and Capillary Dilatation.—We have seen that histamine has a powerful dilator effect on the capillaries, and also that there is some evidence in support of the opinion that substances similar in their action to histamine are produced in quantity by gross damage to large masses of tissues, and play a part in the production of surgical shock.

Is it possible that the production of such substances in proportionally smaller amount may be a consequence of even mild injury to the tissues, and may be responsible for the transitory dilatation of the skin vessels which follows on such very limited damage as is done in evoking the red *tache ?*

Lewis has shown that the skin of all young subjects is liable to form wheals following flares as a result of firm stroking pressure with a blunt instrument; further experiments by Lewis and Grant supplied the explanation of this wheal formation, for they found that similar flares and wheals could be imitated by puncturing the skin with fine glass capillaries filled with histamine solution. The results of the histamine inoculation and of firm stroking pressure were so similar as regards their course, time relations, and consequence that it seems clear that they have a common explanation, namely, that a substance having a histamine-like action is liberated in the skin as a result of the mechanical treatment. Further, they showed that similar explanations appear to apply to the causation of skin reactions, œdema, and finally wheals and blisters, in response to such other

injuries as are caused by scratches, pin-pricks or the application of heat and cold, electricity or, with certain exceptions due to the longer latent period, to the effects of ultra-violet light, X-rays and radium. The introduction of wheal fluid into normal skin was shown sometimes to lead to the formation of a second wheal.

The portion of the skin from which the so-called H-substance is formed appears to be the living cells of the deeper layers of the epidermis, which indeed actually contain, or rather yield, considerable amounts of histamine.

We might, then, regard the production of " H-substance " by the tissues as a physiological process liable to be induced even by the limited amounts of damage met with in daily life, or perhaps even set free from the tissues by nervous action as in the axon reflexes ; and we might regard adrenaline as the normal antagonist to this action, preventing its consequences from reaching undue proportions.

It would seem likely that the H-substance, or histamine itself, exists pre-formed in most mammalian tissues, and is liberated from the cells and thus enabled to produce its local effects on the blood vessels, when the cells are damaged or appropriately stimulated. The largest amounts of histamine are obtained from lung tissue (Best, Dale, Dudley and Thorpe), and, since it is difficult to ascribe to it any vaso-dilator function in that situation, it has been surmised by Dale that the lungs might be a site for histamine destruction. In this connection it is suggestive that Best and McHenry have quite recently found that the histamine present in fresh lung tissue, or histamine added to it, is very rapidly destroyed.

It is interesting to note that Dale and Dudley have recently succeeded in isolating from spleen tissue both histamine and acetyl choline, which had for some little time both been suspected to be responsible for the depressor action of certain tissue extracts. Best, Dale, Dudley and Thorpe isolated choline and histamine from liver and lung,

while Thorpe found that in muscle the chief depressor substance was histamine. In this connection it is suggestive to note that the actions of acetyl choline on the body resembles that of the parasympathetic nervous system as closely as the effects of adrenaline reproduce those of the sympathetic supply. Hunt has shown that the distribution of the vasodilator action of acetyl choline in a limb is closely similar to that produced by stimulation of antidromic fibres. It is quite conceivable, therefore, that, just as the effects of parasympathetic stimulation in other parts (*e.g.*, vagus on the heart or intestine) are suspected to be due to the liberation of minute amounts of acetyl choline, so also in the case of excitations of antidromic fibres, the vascular and other responses may be due to a similar local liberation of the same substance.

The Permeability of the Tissues for Oxygen.—The rate at which oxygen reaches the tissues will depend not only on the oxygen pressure head, but also on the *coefficient of diffusion of oxygen* through the intervening tissues. This latter has been determined by Krogh, who defines it as the volume of gas diffusing through 1 sq. cm. and 1 μ thickness in one minute at a pressure head of 1 atmosphere. It increases with rise of temperature about 1 per cent. per degree, taking the value at 20° C. as unity, and is much slower for animal tissues than for water, or even gelatine.

It is clear that in any tissue which is itself using oxygen the oxygen tension will be lower at any particular point the further that point is from the source of oxygen, *i.e.*, from the nearest capillary, and will be proportional to the diffusion rate (d) of oxygen through the tissue, and inversely as the rate of gas exchange of the tissue (p). From these results, assuming that (in muscle, for example, where the capillaries are rectilinear) each capillary supplies a cylinder of tissue of radius R, he establishes a formula which gives the pressure gradient which would be necessary fully to supply the tissue with oxygen to the limits (R) of the district served by the

capillary of radius r. This pressure ($T_o - T_r$) is obviously $\propto p/d$, and the equation is

$$T_o - T_r = p/d \left(1{\cdot}15\ R^2 \log \frac{R}{r} - \frac{R^2 - r^2}{4}\right).$$

T_o is the oxygen pressure in the capillary, and T_r that at the farthest point from it in the surrounding zone, *i.e.*, at the distance R. The driving force is To — Tr. If this required driving force be greater than the oxygen tension of the venous blood, it is obvious that some parts of the tissue must suffer from oxygen lack (which seems to be the case in frog's muscle at rest), while, if it is smaller, the oxygen tension must be positive throughout the tissue. As already mentioned, Krogh has shown that the distance (R), which is proportional to the distance between the capillaries, varies according to the oxygen requirement of the tissues owing to the opening up of previously constricted capillaries with the onset of oxygen want. If the number of patent capillaries were not reduced in states of rest, the pressure head necessary for supply would be very small indeed (0·1 to 0·4 mm. Hg), but, owing to the physiological variation in the numbers of capillaries, the gradient required in states of rest must, because the capillaries are further apart, be much greater than that needed during work.

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CHAPTER IV

THE CARRIAGE OF CARBON DIOXIDE BY THE BLOOD

Carbon dioxide is readily soluble in water, and the amount dissolved at any given pressure of the gas can be readily found from the known coefficient of solubility at the particular temperature. Thus at 38° C. 100 c.c. of water will dissolve 54·6 c.c. of CO_2 (measured at N.T.P.) when the pressure of the gas is 1 atm. (760 mm. Hg). Hence at 40 mm. Hg pressure of CO_2, which is about the pressure of the gas in arterial blood, water at 38° will dissolve about 2·9 volumes per cent. of carbon dioxide; for whole blood under similar conditions somewhat less CO_2 would be held in physical solution, viz. about 2·7 volumes per 100 c.c.

Now it has long been known that blood as drawn from the body contains as much as 50 to 60 volumes of carbon dioxide held in such a state as to be removable by exposure of the blood to a vacuum, and we have now to inquire how it is that blood is capable of holding amounts of carbon dioxide which are so very much greater than can be held in physical solution. A further fact which has also been long known is that this carbon dioxide can not only be withdrawn from blood by a vacuum, but can also be expelled from it by the addition of sufficient amounts of strong acids. This suggests at once that the carbon dioxide is held in the blood in the form of a chemical combination with a base to form a bicarbonate or carbonate, from which union it is expelled when a more powerful acid enters into competition with it for possession of the base, or when the blood is exposed to a vacuum. It should further be noted that after all possible

carbon dioxide has been drawn from blood by exposure to a vacuum no more can be driven off on addition of a strong acid. One method of expulsion is as good as the other so far as the total yield of CO_2 from whole blood is concerned. Researches during the past ten years have done much to elucidate the means by which carbon dioxide is carried by the blood, and to show that an interesting chemical mechanism is concerned in it. Perhaps the most valuable information has been obtained by the study of the CO_2 dissociation curves of blood, and of somewhat similar chemical systems.

CO_2 Dissociation Curves of Blood and of Separated Plasma

Accurate dissociation curves were obtained by Christiansen, Douglas and Haldane (Fig. 32, p. 95), and have since been repeated by many others, with the improved technique now available.

The technique of the construction of a carbon-dioxide dissociation curve is in general principles very simple. The sample of blood is usually drawn from a vein, and coagulation is prevented by the addition of potassium oxalate (about 0·2 per cent.) together with about 0·05 per cent. of sodium fluoride : the latter substance is added to prevent conversion of the blood glucose into lactic acid, which, by combining with base, would lower the carbon-dioxide combining power of the blood if kept for long at body temperature.

About 3 to 5 c.c. of the blood is then placed in a suitable saturating chamber. The saturator used by Barcroft is convenient ; this is a glass cylinder of about 300 c.c. capacity, with a neck at one end, while at the other end the cylinder is drawn out to a narrow portion of a few cubic centimetres capacity, bearing a three-way tap. The saturator thus resembles a separating funnel with a three-way tap. After introduction of the blood the neck of the funnel is closed with a rubber stopper, which may conveniently also carry a three-way glass tap.

Suppose now that we wish to plot a point on the curve corresponding to a carbon-dioxide pressure of, say, 10 per cent. of an atmosphere. This would mean that it would be necessary to add to the 300 c.c. of air about 30 c.c. of carbon dioxide. The saturator is connected to a filter pump, and partially evacuated. The use of a mercury manometer is convenient as a guide to the degree of exhaustion reached ; for the example under consideration about one-tenth atmosphere of vacuum would suffice. The

tap is now closed, and the saturator connected with a measuring burette containing carbon dioxide over mercury. The required amount of the gas is allowed to enter the partially exhausted cylinder, and then the gas burette is disconnected. There should still be a partial vacuum in the saturator, and this is now filled by momentarily turning the tap to the atmosphere, so that air enters and equalises the pressure.

The saturator is now immersed in a water-bath at the required temperature (38°), and is rotated in a horizontal position, so that the blood spreads in a thin film over the inside of the cylinder. After about five minutes of this spinning, the saturator is tilted so that the blood runs to the narrow end, and the tap at the other end just emerges from the surface of the water in the bath. This tap is now momentarily turned, and thus the pressure in the saturator, which has risen owing to expansion from rise of temperature, is equalised by escape of gas. The tap is now closed, the tonometer restored to its horizontal position beneath the water, and the rotation continued for a further fifteen to twenty minutes. After this time the saturator is held vertically in the bath, so that the blood runs down to the narrow end, while the top tap just emerges from the water. This tap is now connected to a small pattern Haldane's gas analysis apparatus, and a sample of the gas in the saturator is withdrawn, with proper precautions, for analysis. The analysis gives the exact carbon-dioxide content of the gas mixture in the saturator.

Immediately after the withdrawal of the gas sample the saturator is opened by removal of the stopper, a pipette is passed down, and a sample of 1 or 2 c.c. of the blood taken. In this blood the total carbon dioxide is determined by any suitable method, *e.g.*, by means of the small blood-gas analysis apparatus of Haldane or Van Slyke.

When the carbon-dioxide pressure required is small, the CO_2 of the blood is first removed nearly completely by warming *in vacuo*. Another way of reaching small carbon-dioxide tensions is to place the blood in air, equilibrate with it in the saturator (when CO_2 will be given off to the air) and then make an analysis of the gas contained in the saturator. If this is not low enough in CO_2, the gas is blown out with a hand pump, and a new equilibration carried out. When a pressure of about 40 mm. is required, the saturator is filled with alveolar air by blowing into it.

In this way we get as many points on the curve as we choose to make. At each point we have the carbon-dioxide pressure of the gas mixture with which the blood is in equilibrium, and also the amount of carbon dioxide which the blood holds at that pressure. The results are then plotted in the form of a curve.

The method just described gives the carbon-dioxide dissociation curve of oxygenated blood. If we require that for reduced blood, the procedure is the same except that we start by reducing the blood and then filling the saturator with hydrogen or nitrogen instead of with air.

In the calculation of gas pressure we must remember to deduct the tension of aqueous vapour at the equilibration temperature. At 38° C. this is 49 mm. Hg. Suppose that at 38° the gas contained 4·2 per cent. CO_2, and that the barometric pressure at the time was 758 mm. Then the effective gas pressure in the saturator would be $758 - 49 = 709$ mm., and the partial pressure of

$$CO_2 = \frac{4{\cdot}2 \times 709}{100} \text{ mm.} = 29{\cdot}7 \text{ mm. Hg.}$$

The simplest explanation that springs to the mind is that

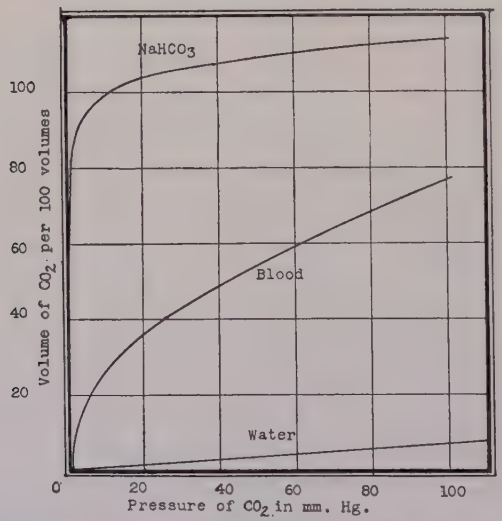

FIG. 29.—Comparison between the CO_2 dissociation curves of blood and of sodium bicarbonate solution of a concentration of 0·0484 N. The amounts of CO_2 held in physical solution in water are also shown (lowest line). (Redrawn from data by Parsons, *Journal of Physiology*, 1919, **53**, p. 57.)

the carbon dioxide in the blood is held in combination with bases, such as sodium, in the form of bicarbonates. It is now generally admitted that this is so, though this is by no means the whole story. If we compare, for instance, the dissociation curve of CO_2 for blood with that of a solution of sodium bicarbonate containing the same amount of available base, as is shown in Fig. 29, it is evident that there is a very great

difference between them. For whereas all the carbon dioxide is removed from the blood when the pressure of CO_2 is reduced to zero (*i.e.*, in a vacuum), it is impossible to remove from the bicarbonate solution more carbon dioxide than corresponds to its conversion to carbonate, according to the equation—

$$2NaHCO_3 = Na_2CO_3 + CO_2 + H_2O;$$

i.e., from an aqueous bicarbonate solution only half as much carbon dioxide can be expelled by a vacuum as by addition of a strong acid :—

$$2NaHCO_3 + 2HCl = 2NaCl + 2CO_2 + 2H_2O.$$

If, therefore, we have reason to believe that the carbon dioxide *is* fixed in the form of bicarbonates in the blood, then we must also have in the blood some substance which, while not interfering with the taking up of carbon dioxide by the base when the blood is exposed to increasing pressures of carbon dioxide, will yet act as an acid does in allowing withdrawal of carbon dioxide, ultimately to completion, as the partial pressure of CO_2 falls. As a preliminary to the search for the substance which causes this remarkable behaviour in blood, let us examine the dissociation curves of blood plasma to see if it behaves as blood does. A glance at Fig. 30 tells us at once that separated plasma behaves less like blood than like an aqueous bicarbonate solution. This is confirmed by the well-known fact that, although all the CO_2 can be removed from blood by a vacuum, this cannot be done with blood plasma ; after plasma has been thoroughly pumped out *in vacuo*, addition of acid can still set free carbon dioxide from it. It is evident, therefore, that the carrying power of blood for carbon dioxide is largely *conditioned by some substance contained in the corpuscles.* It is the corpuscles which *in vacuo* act like the acid, expelling carbon dioxide. This can be proved by the simple experiment of mixing thoroughly well-evacuated plasma with corpuscles, when CO_2 is at once evolved on evacuation.

The Alkali Reserve.—The carbon dioxide which is bound in the plasma is unquestionably present there in the form of an inorganic (chiefly sodium) bicarbonate. This is shown by the fact that the amount of carbon dioxide which can be expelled from the plasma by addition of a strong acid, like sulphuric acid, agrees with the amount of base found by

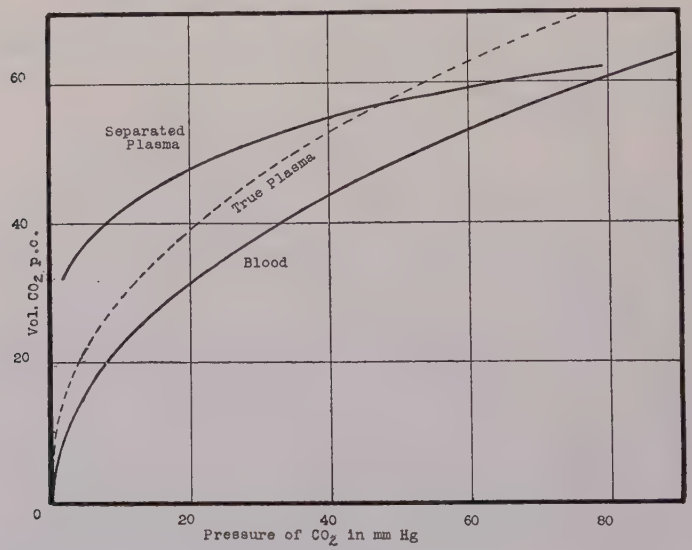

FIG. 30.—Comparison between the CO_2 dissociation curves of blood and of separated plasma and true plasma from the same blood. (Redrawn from data by Joffe and Poulton, *Journal of Physiology*, 1920, **54**, p. 129.)

titration with an acid, and further all this bicarbonate can be removed as such by dialysis. If plasma is saturated with carbon dioxide at atmospheric pressure, practically all the available base of the plasma will be present in the form of sodium bicarbonate. The amount of bicarbonate present in the plasma under any circumstances can readily be determined, and forms a measure of the available base present in that plasma. This is sometimes expressed in the form

of the volume of carbon dioxide held in the combined state by 100 volumes of plasma, and is called the "alkali reserve" of that plasma.[1] It measures the amount of bicarbonate in the plasma.

True Plasma.—The name "true plasma" is given to plasma drawn direct from the blood without any change in its gas content. Specimens of true plasma are in practice obtained by centrifuging the blood under a layer of liquid paraffin to hinder exchange of gases at the surface, and then pipetting off the plasma layer. This represents the plasma which is in a state of equilibrium with the corpuscles of the blood.

Now, if blood be saturated with gas mixtures containing different pressures of carbon dioxide, it is a simple matter to separate off samples of true plasma corresponding to each pressure of CO_2, to determine their bicarbonate content, and thus to obtain a curve which may be called the dissociation curve of true plasma. Such a curve represents the carbon dioxide content of plasma which is in equilibrium with the corpuscles over the range of pressures of carbon dioxide which are under investigation.

When this is done, it at once becomes evident that the true plasma behaves in a way entirely different from the separated plasma discussed above. The following table (p. 38) and Fig. 30 illustrate this difference.

The true plasma shows a curve which has much the same slope as that for blood, but at a higher level; the plasma of blood when in equilibrium with its corpuscles has sometimes a higher percentage bicarbonate content not only than separated plasma, but even than the whole blood. This is because the carbon dioxide-fixing power of blood is the sum of that of the corpuscles and that of the true plasma. If the

[1] Strictly speaking, the "alkali reserve" is defined as the volume per cent. of CO_2 yielded by the plasma on treatment with acid after the plasma has been previously brought into equilibrium with a gas mixture containing CO_2 at 40 mm. pressure (alveolar air).

Curves for Separated and True Plasma.

(From Joffe and Poulton.)

Separated Plasma. Drawn from Blood at 44·5 mm. CO_2 and then brought into equilibrium with the CO_2 pressures given below.		True Plasma. Separated from Blood in equilibrium with the various CO_2 pressures given below.	
CO_2 pressure, mm.	CO_2 in 100 c.c.	CO_2 pressure, mm.	CO_2 in 100 c.c.
	c.c.		c.c.
1·8	31·8	—	—
12·2	43·7	10·0	29·9
21·1	48·3	20·0	39·3
—	—	30·0	46·8
42·0	53·7	40·0	52·8
67·2	59·6	55·0	61·0
—	—	70·0	67·8
79·0	63·0	90·0	74·1

true plasma contains per 100 volumes more fixed CO_2 than blood does, then evidently 100 volumes of corpuscles must be capable of actually carrying considerably less carbon dioxide than an equal volume of blood. The next table shows that this is so, and that when the amounts of fixed CO_2 carried by corpuscles and true plasma contained in 100 c.c. of blood are calculated, rather more of the load is actually carried in the plasma

Partition of Carbon Dioxide Carriage between Blood Plasma and Red Cells.

(From Joffe and Poulton.)

At the given pressures of carbon dioxide the true plasma and the corpuscles present in 100 c.c. blood carry the amounts of CO_2 shown.

CO_2 pressure, mm. Hg.	Volumes of Carbon Dioxide carried by		Total by 100 c.c. Blood.
	Plasma (49·07 c.c.) of 100 c.c. Blood.	Corpuscles (50·93 c.c.) of 100 c.c. Blood.	
	c.c.	c.c.	c.c.
10	14·7	7·7	22·4
20	19·3	12·5	31·8
30	23·0	16·0	39·0
40	25·9	19·1	45·0
55	29·9	22·4	52·3
70	33·3	25·2	58·5
90	36·4	27·9	64·3

Relation of CO_2 Content of Whole Blood to that of True Plasma.—While it is often desirable to know the carbon

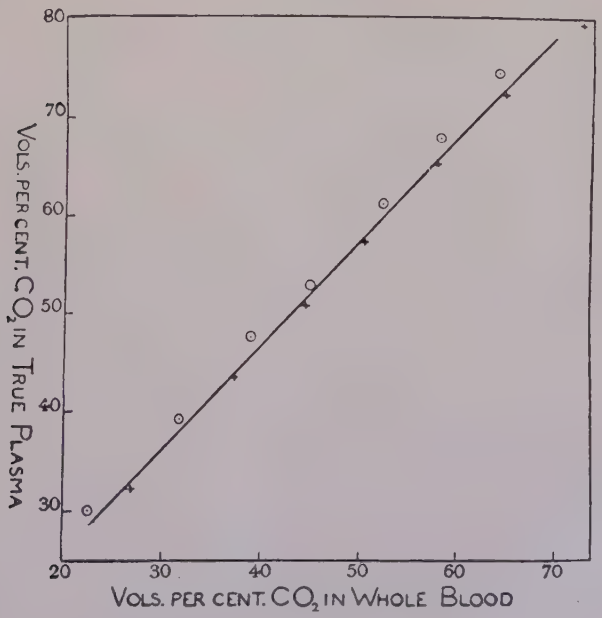

FIG. 31. (From Report No. 72 Medical Research Council.)

dioxide content of the true plasma, it is not always convenient to prepare the plasma for this determination. The amount of CO_2 in the true plasma was found by Campbell and Poulton to be greater than that in whole blood, but they found that for ordinary ranges of CO_2 pressure there is a linear relationship between the two. Hence, if the CO_2 content of whole blood is known, that of the true plasma can be determined by reference to a graph showing the relationship between the two quantities. Such a graph is given in Fig. 31.

The Ionic Interchange (Hamburger Phenomenon) in Blood.—When we compare the behaviour of true and separated plasmas, it is evident that the true plasma is enabled, by the presence of the corpuscles, to increase its available base (alkali reserve), and so to carry more carbon dioxide in the form of bicarbonate at a given carbon dioxide pressure than separated plasma can do. The carbon dioxide is, therefore, carried in the plasma, but in virtue of the presence of corpuscles. This phenomenon was first clearly described by Zuntz in 1867, who believed that when blood is treated with CO_2 " a carbonic acid carrier passes from the corpuscles into the serum (plasma) in amount approximately proportional to the increase in the CO_2 content."

We know now that this is not exactly what occurs. The present-day explanation is based on physico-chemical laws. There are in the corpuscles certain weak acids; these are (1) hæmoglobin, (2) phosphates, such as KH_2PO_4. The bases present in the corpuscles may combine with these acids, but can be removed again by a stronger acid if such is present to compete. The envelope of the red cells is not permeable to hæmoglobin or to cations such as K; it is, however, permeable to anions and to carbonic acid, and when blood is treated with carbon dioxide the following chain of events takes place :—

First, the carbon dioxide passes into solution to form carbonic acid :—

(1) $$CO_2 + H_2O = H_2CO_3,$$

and some of this enters the corpuscles. Here it reacts with the potassium salt of hæmoglobin :—

(2) $$H_2CO_3 + KHb = KHCO_3 + HHb.$$

We now have the following ions present :—

In the plasma, $Na^{\cdot}$ and Cl'.
In the corpuscle $K^{\cdot}$, HCO_3', $H^{\cdot}$ and Hb',

and the anions pass through the corpuscular membrane until an equilibrium is reached, *i.e.*, Cl' enters the corpuscle and HCO_3' leaves it.

Owing to the decomposition of KHb to form a larger number of osmotically active ions, there is an increase of the osmotic pressure of the contents of the corpuscle, which therefore swells.

The net result of these changes is that when blood is treated with carbon dioxide—

(1) The chloride content of the plasma is diminished ;
(2) The bicarbonate content of the plasma and of the corpuscles is increased ;
(3) The chloride content of the corpuscles is increased ;
(4) The actual metallic cation content of the corpuscles and of the plasma is unaltered.
(5) Water enters the corpuscles.

There are other subsidiary changes, to which reference will be made later, but at present we are only discussing the main one, by reason of which the bicarbonate content of the true plasma is increased.

The figures on p. 92 illustrate the change which occurred in the true plasmas of two bloods exposed to oxygen containing different amounts of CO_2 :—

Chloride and Bicarbonate Shifts in Plasma.

Experiment.	Partial Pressure of CO_2 to which Blood was exposed.	True Plasma.	
		Chloride.	Bicarbonate.
(1)	6·0 mm.	0·615 per cent. NaCl [0·105 M]	44·3 vols. CO_2 per cent. [0·0195 M]
	91·0 ,,	0·585 per cent. NaCl [0·10 M]	78·6 vols. CO_2 per cent. [0·0345 M]
(2)	3·8 mm.	0·650 per cent. NaCl [0·111 M]	52·6 vols. CO_2 per cent. [0·023 M]
	66·0 ,,	0·595 per cent. NaCl [0·102 M]	84·5 vols. CO_2 per cent. [0·038 M]

The figures in square brackets give the concentrations of chloride and bicarbonate in gram molecules per litre. When these are compared it is clear that the bicarbonate shows a greater change in molarity than the chloride. This is because the reaction described above is not the only one which takes place; if it were, the changes in molarity of chloride and bicarbonate would be equal.

Subsidiary Phenomena in CO_2 Carriage.—Of the other reactions, an important one takes place in the plasma alone, and is due to similar reactions to those in which the corpuscles are involved. The plasma proteins are able, like the hæmoglobin of the corpuscles, to act as feeble acids and combine with bases which can be removed from them again by an acid stronger than themselves; so we have such reactions as the following taking place in the plasma :—

$$\underset{\text{(Salt.)}}{\text{Na protein}} + H_2CO_3 = NaHCO_3 + \underset{\text{(Acid.)}}{\text{H protein}};$$

and it is the occurrence of reactions of this nature which explains why the dissociation curve of separated plasma does not exactly resemble that of a bicarbonate solution. At low pressures of CO_2 the available base of the plasma is

present to some extent in combination with protein ; as the carbon dioxide pressure is raised this sodium protein compound is decomposed, so that some of the base becomes available to combine with carbon dioxide.

Another factor which adds to the carbon dioxide-carrying power of the blood is the fact that the following reaction also occurs in the corpuscles :—

$$H_2CO_3 + K_2HPO_4 = KH_2PO_4 + KHCO_3 ;$$

and this explains why considerable amounts of carbon dioxide are found to be present in the corpuscles.

The Donnan Equilibrium.—The diffusion of anions across the corpuscular membrane does not lead to equalisation ; this is because of a phenomenon known as the Donnan membrane equilibrium, which is met with in all cases where a membrane separates two solutions in one of which is a non-diffusible ion. In this case the non-diffusible ion is that of the colloid hæmoglobin. Under such circumstances the diffusible ions are distributed so that the products of diffusible anions and cations on the two sides are equal. Applying this to the inside and outside of the corpuscle we should have

$$\underset{\text{(cell)}}{\text{Cations}} \times \underset{\text{(cell)}}{\text{anions}} = \underset{\text{(plasma)}}{\text{Cations}} \times \underset{\text{(plasma)}}{\text{anions}}$$

or,
$$\frac{\text{Cations (cell)}}{\text{Cations (plasma)}} = \frac{\text{Anions (plasma)}}{\text{Anions (cell)}}$$

or taking specific ions we should find that

$$\frac{HCO_3'(c)}{HCO_3'(p)} = \frac{Cl'(c)}{Cl'(p)} = \frac{H\cdot(p)}{H\cdot(c)} \text{etc.}$$

(Since the cell is impermeable to cations, these do not alter in reaching the equilibrium, with the exception of $H\cdot$, which is derived from H_2CO_3, which can penetrate.)

This ratio of anions (c)/anions (p) is called the Donnan ratio, and it has been shown by Van Slyke that this falls

below unity by an amount proportional to the amount of base as hæmoglobinate in the cells, *i.e.*,

$$\frac{\text{Anions (cells)}}{\text{Anions (plasma)}} = 1 - \frac{\text{KHb (cells)}}{\text{Anions (plasma)} \times 2}$$

If we take the anions of plasma as about 150 milliequivalents, and KHb cells as about 75 milliequivalents, we find that the ratio = about 0·75.

We thus see that the blood carries CO_2 by reason of the properties of hæmoglobin, and that the corpuscular membrane plays an important part.

Carbon Dioxide Relations of Oxygenated and Reduced Blood.—It was shown by Christiansen, Douglas, and Haldane that at equal partial pressures of carbon dioxide a greater amount of it is fixed by reduced than by oxygenated blood. In other words, oxygen tends to displace carbon dioxide from the blood, just as carbon dioxide is known to displace oxygen. They explained this phenomenon on the hypothesis that oxyhæmoglobin is a stronger acid than reduced hæmoglobin, and all subsequent work has confirmed this belief.

Fig. 32 reproduces from the paper of Christiansen, Douglas, and Haldane the curve for oxygenated and reduced blood, and shows that at pressures of carbon dioxide which fall within physiological limits, from 5 to 6 volumes per cent. more of CO_2 is taken up by reduced than by oxygenated blood.

The importance of this observation lies in the fact that, during its passage along the systemic capillaries, not only is carbon dioxide added to the blood, but also oxygen is withdrawn from it, and *vice versâ* in passing the lungs.

When two acids compete for possession of a base which is present in amount insufficient to satisfy both, the base will be distributed between the acids according to their strengths, the stronger acid seizing the greater part of the base. Now, if oxyhæmoglobin is a stronger acid than reduced hæmoglobin, we may consider the following changes to occur when

carbon dioxide is added to the blood and at the same time oxygen is withdrawn from it :—

First, we have the oxyhæmoglobin present chiefly as its potassium salt. Oxygen leaves the corpuscle, let us suppose, completely, and this weakens the acidic properties of the

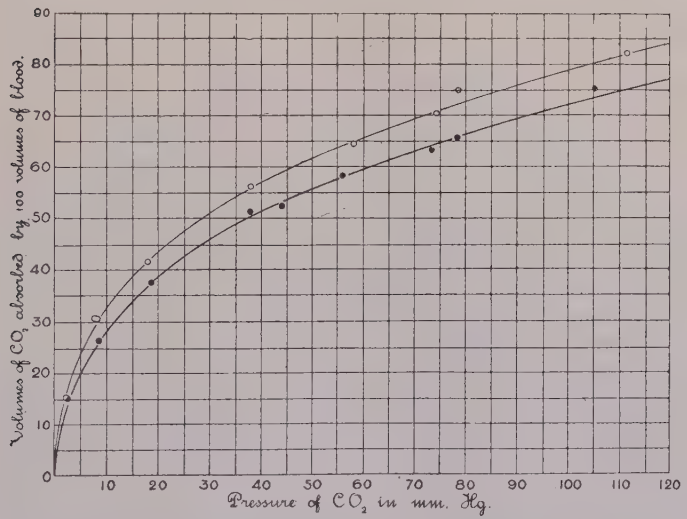

FIG. 32.—Carbon dioxide dissociation curves of blood, lower curve in presence of air and CO_2, upper curve in presence of hydrogen and CO_2. (Christiansen, Douglas, and Haldane, *Journal of Physiology*, 48.)

hæmoglobin, so that it holds the combined base less firmly. At the same time the carbon dioxide enters the corpuscle from the plasma, as we have previously explained, and against this the feebly acidic hæmoglobin can compete very little ; it therefore gives up its base more readily than it would if it were oxygenated ; hence the reaction

$$KHb + H_2CO_3 \rightarrow HHb + KHCO_3$$

proceeds much more nearly to completion and, in consequence, the ionic interchange with the plasma is more

extensive, and more $NaHCO_3$ is formed in it, while the amount of bicarbonate stored in the corpuscles is also greater than it would be if oxygen had not left the corpuscle. Exactly the reverse changes take place in the conversion of venous into arterial blood.[1]

Physiological CO_2 Curve. It is of interest in this connec-

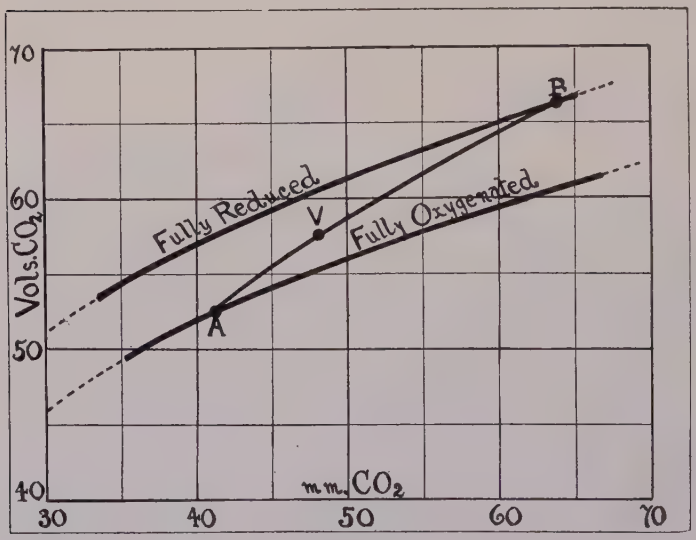

FIG. 33.

tion to attempt to form an opinion of the carbon dioxide absorption curve of blood under the conditions which prevail in the body. Such a curve (for an R.Q. of about 0·8) is shown in Fig. 33, in which portions of the carbon dioxide

[1] The magnitude of this effect of alteration of the oxygenation of the oxyhæmoglobin is much greater than might be supposed. Peters, Barr, and Rule have shown that at CO_2 tensions between 30 and 70 mm. Hg the increase of CO_2 content from this cause = 0·35 × the oxygen deficit. In fully reduced blood of 18·5 per cent. oxygen capacity it is, therefore, 0·35 × 18·5 = 6·3 volumes per cent. From this Van Slyke has calculated that, when the R.Q. is 0·8 the rise of carbon dioxide content of blood produced by a given fairly large rise of CO_2 pressure is 78 per cent. greater than it would otherwise be.

curves for fully reduced and fully oxidised blood are shown on a large scale. The curve AVB represents the physiological carbon dioxide absorption curve. At the point A, which may be called the "arterial point," since it represents the condition in arterial blood, the fully oxygenated blood at 41 mm. CO_2 contains 52·5 volumes CO_2 per cent. The point B represents the condition if the blood were fully reduced (*i.e.*, had lost in this case 17·5 volumes of oxygen per cent., and therefore gained 17·5 × 0·8 c.c., *i.e.*, 14 c.c. of CO_2); it would then contain at the pressure of CO_2 of 64 mm., not 61 per cent. of CO_2 (as would be the case had there been the same change of CO_2 pressure without loss of oxygen), but 66·5 per cent. Usually the blood does not nearly reach the fully reduced state in passing through the capillaries, but would in most cases stop at a point about in the position indicated by V in the figure. This corresponds to a gain of 5 volumes of CO_2, and therefore a loss (since the R.Q. is assumed to be 0·8) of about 6·3 c.c. of oxygen. The point V is thus a typical venous point. It will be seen that in this instance at least half the increase in CO_2 content of the blood is due to the simultaneous reduction of oxygen content.

To sum up the whole question, we can say that when carbon dioxide is added to the blood it mostly combines directly or indirectly with base which is set free from combination with hæmoglobin. According to calculations by Van Slyke from various available data, about 80 to 85 per cent. of the base set free is liberated by the change of oxyhæmoglobin to reduced hæmoglobin. The direct "buffer" action of oxyhæmoglobin without simultaneous reduction, as in the simple Hamburger ionic interchange, probably accounts for about 10 per cent. of the change. Hæmoglobin is therefore the chief carbon dioxide-"carrier" of the blood, though under ordinary conditions it does not appear to combine with it directly.

Direct Union of Carbon Dioxide with Hæmoglobin.—It was

held by Bohr that hæmoglobin can combine directly not only with oxygen, but also with carbon dioxide, and Buckmaster has shown that this is undoubtedly true. It might seem at first sight strange that hæmoglobin may thus function as a base. The answer is that the property of hæmoglobin, like that of other proteins, depends on the hydrogen ion concentration of the solution in which it exists. It is an *amphoteric* substance, and in a sufficiently alkaline solution functions as an acid, while in a sufficiently acid solution it functions as a base. At some intermediate reaction the protein behaves indifferently. Campbell and Poulton have given grounds for the belief that the " isoelectric " or indifferent point of hæmoglobin is at a hydrogen ion concentration of $10^{-6.8}$, that is to say, definitely on the acid side of neutrality. Hence, in blood under ordinary conditions, hæmoglobin behaves as an acid, because the hydrogen ion concentration of the blood is always less than (*i.e.*, less acid than) the isoelectric point of Hb. In dialysed solutions of hæmoglobin, however, the reaction is neutral to begin with, and as soon as carbon dioxide is added the result is a solution acid enough to impart basic properties to the hæmoglobin. The explanation of the matter then seems to be that, while hæmoglobin is able to combine with carbon dioxide under such conditions, these are never likely to be realised in blood. This does not in the least alter our statement, made a little way back, that hæmoglobin is the most important constituent of the blood in carrying carbon dioxide. Let it be repeated that in blood hæmoglobin functions as an acid capable of combining with base. When carbon dioxide is added to, or oxygen removed from, the blood, some of this base is liberated from the hæmoglobin, and either (1) combines with carbon dioxide entering the corpuscle or (2) combines with some other acid in the corpuscle as a result of the ionic interchange dependent upon the addition of carbon dioxide to the plasma, as explained above.

Determination of Carbon Dioxide Pressure in a Blood

Sample.—Use may be made of the carbon dioxide dissociation curves discussed above for the determination of the carbon dioxide pressure of a given sample of blood. Suppose that we wish to know the carbon dioxide pressure in the arterial blood of a given individual. It would first be necessary to draw a sample of his blood (preferably arterial), expose portions of it to different pressures of carbon dioxide, and determine the carbon dioxide content in each case. From these data, a CO_2 dissociation curve would be plotted. A sample of the blood would then be drawn by arterial puncture, without exposure to the air or loss of CO_2, and its CO_2 content determined forthwith. By reference to the curve it could be found what the carbon dioxide pressure must have been for the blood to contain the amount of CO_2 actually found there. If the sample of blood was not fully oxygenated, it would be necessary to utilise the physiological CO_2 absorption curve discussed on p. 96; this would necessitate the use of two CO_2 dissociation curves, one for oxygenated and one for reduced blood. It would also be desirable to know the percentage saturation of the blood with oxygen, in order to confirm the location of the point sought for.

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CHAPTER V

THE REACTION OF THE BLOOD

Hydrogen Ion Concentration.—The electrolytic dissociation theory teaches that pure water is to a small, but definite, extent dissociated into the two equally and oppositely charged ions $H^{\cdot}$ and OH', according to the equilibrium

$$H_2O \rightleftharpoons H^{\cdot} + OH'.$$

The concentration of hydrogen ions (often expressed *c*H) thus set free is 10^{-7} gram equivalents (*i.e.*, 10^{-7} grams) per litre at 22° C., so that in 10,000,000 litres of water there is 1 gram of hydrogen in the ionic state. Similarly there are 10^{-7} gram equivalents of hydroxyl ion per litre (*i.e.*, 17 grams of OH ion in 10,000,000 litres). Pure water at 22° may thus be regarded as a ten-millionth normal acid, and also as a ten-millionth normal alkali solution. At 37° C. the concentration of H ions is $1{\cdot}76 \times 10^{-7}$.

The law of mass action tells us that in such an equilibrium as the above the product of the concentration of the two ions is a constant; in water at 22°, since the concentration (in gram-ion equivalents per litre) of each ion is 10^{-7}, the product is

$$[H]^{\cdot} \times [OH]' = 10^{-14}.$$

(At 37° C. the product is $3{\cdot}1 \times 10^{-7}$.)

Now acids, *e.g.* HCl, dissociate in water, yielding two ions,

$$HCl \rightleftharpoons H^{\cdot} + Cl',$$

while alkalies likewise yield two ions, *e.g.*,

$$NaOH \rightleftharpoons Na^{\cdot} + OH'.$$

The product of the concentrations of the two ions of water remains unaltered when acid or alkali is added to it, though in the former case the hydrogen ion, and in the latter case the hydroxyl ion, concentration is increased, the other ion being proportionately reduced by a depression of the ionisation reaction of the water. For instance, in 1/10,000 normal solutions of strong acids (such as HCl), the concentration of $H^{\cdot}$ ions is approximately 10^{-4} N., the effect of adding the strongly ionised acid to water being to depress the ionisation of the water itself,

$$H_2O \leftarrow H^{\cdot} + OH',$$

until a fresh equilibrium is reached, at which the OH' ion has a concentration of 10^{-10} normal. The product of the two ions is still 10^{-14}, as in pure water. Similarly in

1/100,000 N. solution of (strong) acid :
$H^{\cdot} = 10^{-5}$; $OH' = 10^{-9}$; $[H] \times [OH] = 10^{-14}$.

1/1,000 N. solution of (strong) acid :
$H^{\cdot} = 10^{-3}$; $OH' = 10^{-11}$; $[H] \times [OH] = 10^{-14}$.

1/100,000 N. solution of (strong) base :
$H^{\cdot} = 10^{-9}$; $OH' = 10^{-5}$; $[H] \times [OH] = 10^{-14}$.

1/10,000 N. solution of (strong) base :
$H^{\cdot} = 10^{-10}$; $OH' = 10^{-4}$; $[H] \times [OH] = 10^{-14}$.

1/1,000 N. solution of (strong) base :
$H^{\cdot} = 10^{-11}$; $OH' = 10^{-3}$; $[H] \times [OH] = 10^{-14}$,

and so on.

It is evident from this that the degree of actual acidity of any aqueous solution, acid or alkaline, can always be expressed in terms of normality in hydrogen ion. When, as in water, the concentration of the hydrogen ion is 10^{-7} (grams per litre), the solution is neutral. Acid solutions have a *c*H greater than 10^{-7}, *e.g.* 10^{-6}, while alkaline solutions have a *c*H smaller than 10^{-7}, *e.g.* 10^{-8}.

The use of negative powers of 10 is a convenience in writing, but it should be borne in mind that these numbers really refer

to normality in hydrogen ion. Thus 1/10,000 normal H ion = N. $\times 10^{-4}$; 1/100,000 normal H ion = N. $\times 10^{-5}$, and so on, as explained above; and similarly 1/2,000 N. H ion = 5/10,000 N. = 5 $\times 10^{-4}$ N.; and 1/5,000 N. = 2/10,000 N. = 2 $\times 10^{-4}$ N.

The *p*H Nomenclature.—In order to avoid the rather cumbersome type of expression referred to above, a simple abbreviated form of it, suggested by Sörensen, is often conventionally employed. In this mode of expression only the negative exponent is written, and this is designated by the symbol *p*H. Instead of expressing a 1/100,000 N. H-ion concentration as $[H]^{\cdot} = 10^{-5}$, one simply writes *p*H 5·0, and instead of writing 2 $\times 10^{-4}$ for a 1/5,000 N. solution, the shorter expression *p*H 3·7 is used (since 2 $\times 10^{-4} = 10^{-3 \cdot 7}$).[1] The reaction of water, in this nomenclature, is *p*H 7·0 (at 22° C.); as the hydrogen ion concentration rises the *p*H number becomes smaller, and, *vice versâ; solutions of* p*H smaller than seven are acid, those larger than seven alkaline.*

1/1,000 N. acid solution (fully ionised) . = *p*H 3·0
1/10,000 N. acid solution (fully ionised) = *p*H 4·0
1/1,000 N. base solution (fully ionised) . = *p*H 11·0
1/10,000 N. base solution (fully ionised) = *p*H 10·0

The hydroxyl ion concentration could, if desired, be expressed by subtracting the *p*H number at 22° from 14, *e.g.*, in water *p*H = 7 and *p*OH = 7: in 1/1,000 N. acid

[1] Conversion from one system of notation into another is effected by the aid of logarithms. With non-fractional indices the logarithm is obvious. Thus in $cH = 1 \times 10^{-7}$, since the log 10^{-7} is $\bar{7}\cdot0$, the *p*H is denoted by 7·0. In other cases simple addition or subtraction is all that is needed.

Thus, to convert

$cH = 0\cdot5 \times 10^{-7}$ into *p*H, we have log $0\cdot5 = \bar{1}\cdot7$, log $10^{-7} = \bar{7}\cdot0$;

i.e., log $0\cdot5 \times 10^{-7} = \bar{1}\cdot7 + \bar{7}\cdot0 =$

$\bar{8}\cdot7 = -8\cdot0 + 0\cdot7 = -7\cdot3$;

i.e., *p*H = 7·3.

Conversely, to convert

*p*H = 7·7 into *c*H,

*p*H = $-\log H = -8\cdot0 + 0\cdot3$ (and since $0\cdot3 = \log 2\cdot0$)

$cH = 2 \times 10^{-8}$.

pH = 3 and pOH = 11 ; but since the reaction of a solution is adequately expressed by its hydrogen ion concentration, and since the pH is a measure of this, there is usually no need to specify the hydroxyl ion concentration in expressing results, so it is customarily not considered. It should be borne in mind, however, that a phenomenon which seems to depend upon hydrogen ion concentration when this notation is used may in reality depend upon the hydroxyl ion.

The relations of acid, neutral, and alkaline solutions at 22° may be expressed in the following simple schematic forms :—

Acidity : $[H]^{\bullet} > 1 \times 10^{-7} > [OH]'$; pH < 7 < pOH.
Neutrality : $[H]^{\bullet} = 1 \times 10^{-7} = [OH]'$; pH = 7 = pOH.
Alkalinity : $[H]^{\bullet} < 1 \times 10^{-7} < [OH]'$; pH > 7 > pOH.

At body temperature (38° C.) neutrality is at cH = 1·83 × 10^{-7}, *i.e.*, at pH 6·74.

Buffers.—If a solution of hydrochloric or other strong acid be neutralised slowly with caustic soda, and the resulting hydrogen ion concentration change plotted graphically, it will be found that the H-ion concentration changes at first in proportion to the amount of base added, until, in the neighbourhood of the neutral point, there is an abrupt fall of the cH (rise of pH), and the solution suddenly becomes alkaline (Fig. 34). An appropriate indicator would show a sharp change in colour when this happened. If 10 c.c. of n/10 HCl be taken, and 9 c.c. of n/10 NaOH added, the pH will be 3. Addition of 2 c.c. more of alkali will alter the pH to pH 11 ; *i.e.*, the cH is reduced to 1/100,000,000th of its former value.

Now let us consider the effect of addition of alkali to a solution of NaH_2PO_4, of which the graph is also shown in Fig. 34. Here, even in the neighbourhood of the neutral point, the addition of 2 c.c. of n/10 alkali causes a relatively small change in the H-ion concentration, viz., from pH 7·0 to pH 7·4, and in other parts of the curve the relations are similar. Evidently, then, solutions such as NaH_2PO_4 are

able to support the addition of considerable quantities of acids or alkalies without there being a rapid change in

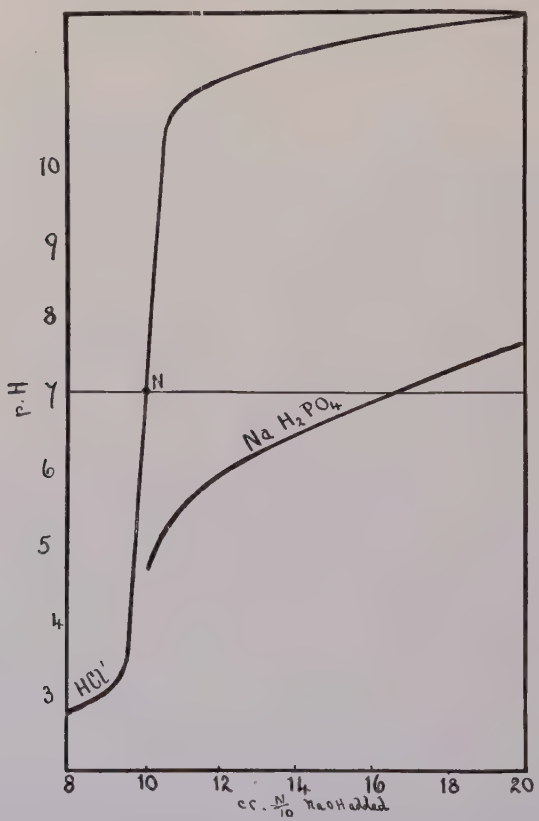

FIG. 34.—Curves of neutralisation of HCl and NaH_2PO_4. In each case 10 c.c. of the m/10 solution are treated with the amounts of n/10 alkali shown in the abscissæ, and the mixture made up to 100 c.c. Ordinates = *p*H; N = neutral point.

the hydrogen ion concentration of the solution. When a coloured indicator is added to such a solution, it only shows a slow change of colour on addition of acid or alkali, because the actual shade of colour given by a particular indicator

depends upon the hydrogen ion concentration of the solution in which it is dissolved.

If water or a solution such as sodium chloride were taken, addition of strong alkali to it would give a curve like the one proceeding upwards from the point marked N, while addition of strong acid would give a curve like the one proceeding downwards from N. A solution of acid sodium phosphate would thus behave very differently from one of an ordinary neutral salt, and would tend to stabilise or "buffer" the solution towards alkalies or acids.

There are various "buffer salts" in the blood, by which its reaction is similarly stabilised and kept constant within remarkably small limits. They are the phosphates (monobasic and dibasic), bicarbonates, and alkali salts of hæmoglobin, and to a smaller extent of other proteins as well. All of them are salts of weak acids, and, as we have already seen, they yield alkali to a stronger acid which comes into competition with them; by so doing the reaction is stabilised.

Theory of Buffer Action.—It has been shown on physicochemical grounds that the hydrogen ion concentration of such a buffered solution depends on the ratio HA/BA, where HA = the free acid, and BA a salt of the acid with a strong base, such as sodium. Thus a sodium bicarbonate solution in equilibrium with carbon dioxide has a hydrogen ion concentration which is determined by the ratio $\frac{H_2CO_3}{NaHCO_3}$.

Since it is the *ratio* between free acid and its sodium salt which determines the hydrogen ion concentration, an important fact in connection with buffered solutions is that their hydrogen ion concentration is unchanged by moderate dilution with water because the ratio is unaltered; in this respect they differ fundamentally from unbuffered solutions having a definitely acid or alkaline reaction, and which on dilution show respectively a fall or a rise of H-ion concentration.

The proof that the hydrogen ion concentration is dependent

upon this ratio belongs to the province of physical chemistry, but may be given briefly as follows :—

We know from the law of mass action that an acid dissociates into its ions according to the equation

$$\frac{[A'] \cdot [H^{\cdot}]}{[AH]} = k \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where $[A']$ is the concentration of the acid anion, $[H^{\cdot}]$ that of the hydrogen ion, $[AH]$ that of the undissociated acid, and k is a constant called the dissociation constant of the acid,

i.e.,
$$[H^{\cdot}] = \frac{k \cdot [AH]}{[A']} \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

The magnitude of the constant k is a measure of the strength of the acid, *i.e.*, of the degree to which it is dissociated into its ions. Some dissociation constants are given below :—

HCl (in normal solution) . . .	3·04
Lactic acid	$1{\cdot}35 \times 10^{-4}$
Acetic acid	$1{\cdot}85 \times 10^{-5}$
Carbonic acid (apparent) . .	$3{\cdot}04 \times 10^{-7}$
Boric acid	$1{\cdot}7 \times 10^{-9}$
Monobasic phosphate (*e.g.*, NaH_2PO_4) .	$1{\cdot}95 \times 10^{-7}$
Dibasic phosphate (*e.g.*, Na_2HPO_4) . .	$3{\cdot}6 \times 10^{-13}$

In the weak acids, it is evident from this table that the degree of ionisation is small. With salts of acids, whether strong or weak, it is quite different, for these are more or less completely dissociated into their ions. If B represent a base, then the degree of dissociation, δ, which occurs when the salt is dissolved in water, and dissociates according to the equation

$$[BA] \rightleftarrows B^{\cdot} + A',$$

will be given by the expression

$$\frac{[A']}{[A'] + [BA]} = \delta.$$

Most salts are dissociated in dilute aqueous solution to the extent of about 80 to 90 per cent., so that $\delta = 0{\cdot}8—0{\cdot}9$.

If, as a first approximation, we assume that the dissociation of a salt of the weak acid is complete, then when in presence of the free weak acid, which is so feebly dissociated, we may take it that the anion concentration of the solution is practically entirely due to the dissociation of the salt, that derived from the acid being disregarded. Hence from equation (2) we have

$$[H^{\cdot}] = k \frac{[HA]}{[BA]} \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

If the degree of dissociation of the salt is appreciably less than unity (*i.e.*, than complete), this must be allowed for, and the expression becomes (from (3))

$$[H^{\cdot}] = \frac{k}{\delta} \cdot \frac{[HA]}{[BA]} \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

We can call k/δ a new constant, k_1, which makes the equation

$$[H^{\cdot}] = k_1 \cdot \frac{[HA]}{[BA]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

which is the proof we started out to find, namely, that *the hydrogen-ion concentration depends on the ratio of free acid to that of its salt.*

This general law applies equally well to a mixture of different buffer salts in presence of their acids. Thus in blood plasma we have bicarbonates, phosphates and protein salts of sodium, but the H-ion concentration of the plasma is, nevertheless, given by the equation

$$[H^{\cdot}] = k_1 \cdot \frac{[H_2CO_3]}{[NaHCO_3]},$$

where k_1 is the special constant determined experimentally for these substances. Since the degree of dissociation is diminished when other salts of the same base are present, it is essential for this purpose to know its value under these conditions, since the value of k_1 will be different. Thus for pure aqueous solutions of $NaHCO_3$ k_1 will be somewhat less than for aqueous solutions of $NaHCO_3$ which also contain NaCl.

Calculation of the H-ion Concentration of Blood.—*The Hasselbalch Formula.*—The H-ion concentration of plasma (*i.e.*, of the blood) is determined by the ratio of free carbonic acid to bicarbonate (*i.e.*, fixed carbonic acid), according to the equation

$$[H^{\cdot}] = k_1 \cdot \frac{[H_2CO_3]}{[BHCO_3]}.$$

If we wish to have the results in terms of the *p*H nomenclature, the equation becomes

$$p\mathrm{H} = p\mathrm{K}_1 + \log \frac{[BHCO_3]}{[H_2CO_3]},$$

$$\text{where } p\mathrm{K}_1 = -\log k_1.$$

Now, since H_2CO_3 is proportional to the dissolved CO_2 (*i.e.*, to the partial pressure of CO_2), we may take it that the concentration of carbonic acid per cent. is proportional to

$$\frac{100\ p\ \alpha}{760},$$

i.e., to the dissolved CO_2, where p = the partial pressure of

CO_2 in millimetres Hg, and α = the solubility of CO_2 in plasma at the particular temperature (0·541 at 38°). Actually the solubility of CO_2 in plasma at 38° C., as estimated by Van Slyke and his collaborators is 0·510, and not 0·541, as estimated by Bohr. The values of pK_1 given here are, however, those found when the solubility is taken at 0·541, and correct results will be obtained with that figure. If the value 0·51 is substituted, then all the values of pK_1 must be reduced by 0·026 to compensate. The $BHCO_3$ is proportional to the amount of fixed CO_2, *i.e.*, to the amount of CO_2 expulsible by a strong acid.

Hence, if we know for any blood the partial pressure of carbon dioxide and the alkali reserve (A.R.) of its true plasma, its H-ion concentration can be at once calculated in terms of pH from the expression

$$pH = pK_1 + \log \frac{7{\cdot}6 \,.\, \text{A.R.}}{0{\cdot}541 \,.\, p}$$

The value of pK_1 in the above formula may be taken as 6·1, but varies somewhat with the bicarbonate content of the plasma, as shown by the following table :—

Values for pK_1 *required for Calculation of* pH *by Hasselbalch's Method.*

Fixed CO_2 per cent. (A.R.).	Values for pK_1 at 38° C.
30	6·11
40	6·09
50	6·07
60	6·06
70	6·054
80	6·05

It is not essential in practice to separate the true plasma from the blood in order to calculate its fixed CO_2 content, since, as shown on p. 89, Campbell and Poulton have found that there is a linear relationship between the CO_2 content of the whole blood and that of the true plasma. A deter-

mination of the CO_2 content of whole blood can, therefore, be employed if the curve shown in Fig. 31 be used to find the CO_2 content of the true plasma.

For finding the CO_2 partial pressure of the blood, all we need to know is the composition of the gas which is in equilibrium with the blood. In laboratory experiments in which the blood is equilibrated in a saturator this is a simple matter (p. 82). For experiments *in vivo* some recognised method of finding the CO_2 pressure, such as the use of the microtonometer or the determination of the "arterial point" from the CO_2 content and dissociation curve, as explained on p. 99, must be employed.

Example.—A sample of arterial blood was drawn, and found to contain 52·5 volumes of CO_2 per cent. Reference to the dissociation curve showed that this corresponded to a CO_2 pressure of about 42 mm. Hg. Reference to the curve relating blood with true plasma gives the fixed CO_2 of the true plasma as 60 volumes per cent. For this content $pk_1 = 6{\cdot}06$.

Hence the *p*H of the blood is

$$6{\cdot}06 + \log \frac{7{\cdot}6 \times 60}{0{\cdot}541 \times 42} = 7{\cdot}36.$$

Methods for Direct Determination of Hydrogen-Ion Concentration

General Principles of the Electrode Method.—If a strip of metal, say silver (or copper), be immersed in water, free ions of the metal carrying a positive electric charge pass out into the water, so that the metal is left with a negative charge proportional to the number of positively charged ions with which it has parted when equilibrium with the solution has been reached. By measurement of the E.M.F. between metal and solution, a measure of the tendency of the metal to pass into (ionic) solution is obtained. If now, instead of water, a solution containing the ions of the metal in question be employed, the tendency for the metal to throw off ions into solution will be opposed by the tendency of the ions already there to be deposited on it, and hence the negative charge on the metal will be less than when pure water was used; the greater the concentration of dissolved ions, the more the

E.M.F. between metal and solution will differ from that between metal and water. By measuring the E.M.F. between solution and metal and applying an appropriate formula it is thus possible to ascertain the concentration of the cations in a solution. The method is of general applicability, and in principle merely consists in immersing a rod of the element in question into the solution containing its ions, the

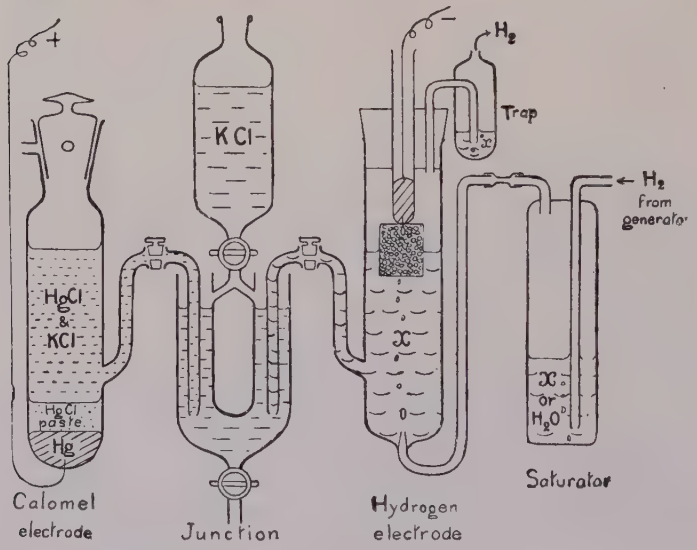

FIG. 35.

concentration of which it is desired to find, and then measuring the E.M.F. set up. The presence of other ions makes no essential difference to the method. Thus, if a mixture of copper and silver sulphate were present in the solution, the concentration of copper and of silver ions would be given by measurements of the E.M.F. with copper and with silver electrodes respectively. In such an electrode an amalgam of copper and mercury could be substituted for pure copper, and a silver amalgam for pure silver.

The Hydrogen Electrode.—In dealing with the hydrogen

cation a special technique is demanded, because the unionised substance is a gas. Hydrogen gas, fortunately, is absorbed in considerable amount by finely divided platinum, and the product obtained behaves electrochemically like solid hydrogen, just as a metallic amalgam behaves like an unamalgamated element.

The principle of the hydrogen electrode, then, is that a wire or plate of platinum is coated with platinum black by electro-deposition, saturated with hydrogen gas, and immersed in the solution the hydrogen ion concentration of which is to be found; the E.M.F. between the platinum-hydrogen element and the solution is then found by putting the hydrogen electrode in series with a standard calomel electrode (Fig. 35) so as to make a complete galvanic cell, the E.M.F. of which is measured in the usual way with a potentiometer. A simple calculation gives the H-ion concentration, or the *p*H of the solution.[1]

Application of the Hydrogen Electrode to Blood.—The use of the hydrogen electrode for the determination of the hydrogen ion concentration of blood is beset with difficulties. A known pressure of pure hydrogen (usually 1 atmosphere) is required in the fluid and in the atmosphere above it; this means that the blood must be deprived of both oxygen and carbon dioxide, which change profoundly alters its reaction; this difficulty can be overcome by separating the "true plasma" and carrying out the electrode measurement on this fluid, the reaction of which is unaffected by removal of oxygen.

The Glass Electrode Method.—When two watery solutions of different H-ion concentrations are separated by a thin

[1] If set up with an n/10 calomel electrode at 38°, and the E.M.F. = E millivolts, then

$$pH = \frac{E - 335{\cdot}5}{61{\cdot}64};$$

e.g., if $E = 785{\cdot}3$ millivolts,

$$pH = \frac{785{\cdot}3 - 335{\cdot}5}{61{\cdot}64} = 7{\cdot}30$$

glass partition (of a certain kind of soft soda glass (Fig. 36)), there is set up between the two solutions an electric potential which depends on their respective hydrogen-ion concentrations. This E.M.F. cannot be determined by means of the usual methods employed for the H-electrode; it can, however, be determined by the use of a delicate electrometer; if the *c*H of one of the solutions is known, that of

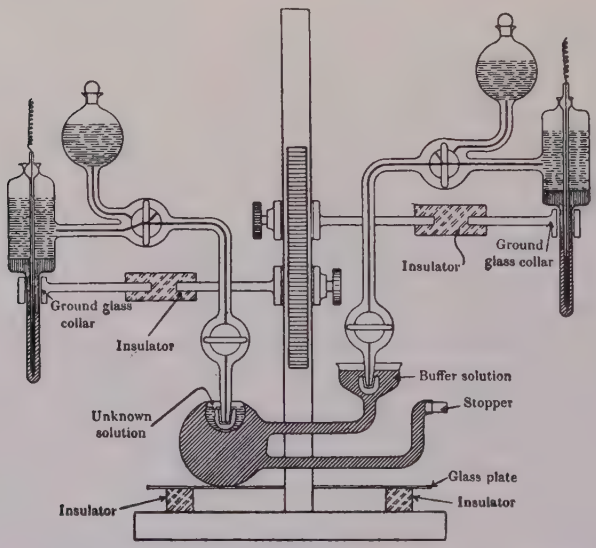

FIG. 36.—Diagram of the glass electrode, with calomel electrodes for leading off, one to electrometer needle, one to potentiometer. (Dr. Tookey Kerridge. From *Journ. Scient. Instruments*, Vol. 3.)

the other can then be calculated. This method, which has recently been developed by Dr. Tookey Kerridge, possesses the advantage that oxygen does not interfere with the result.

Special precautions have to be taken with the electrical arrangements owing to the high resistance of the glass membrane. A potentiometric method is employed for the measurements of the potential, a Lindemann electrometer, the needle of which is observed under the microscope, being

used, instead of a galvanometer, as a null point indicator.[1] The potential thus measured is proportional to the ratio of

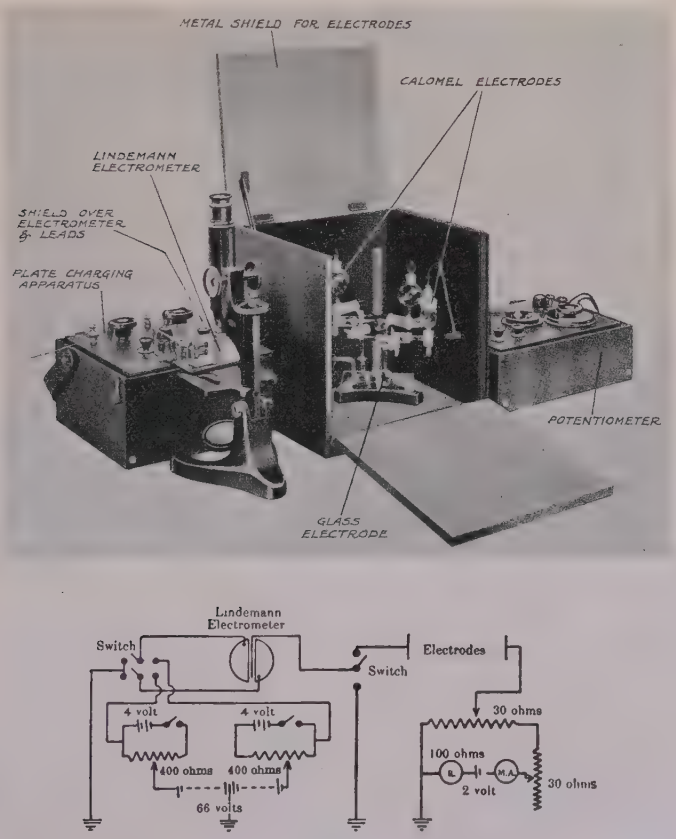

FIG. 37.—Arrangement of glass electrode circuit. Below the plate-charging and potentiometer circuits. (Courtesy of Dr. Kerridge.)

the logarithms of the hydrogen-ion concentrations of the two liquids, according to the Nernst formula.

[1] The apparatus is made by the Cambridge Scientific Apparatus Co.

$$E = \frac{RT}{nF} \log_e \frac{cH_s}{cH_x}$$

$$= \cdot 0577 \log_{10} \frac{cH_s}{cH_x} \text{ at } 18^\circ \text{ C}$$

$$= \cdot 0577 \, (pH_x - pH_s) \text{ at } 18^\circ \text{ C},$$

where

E = measured potential (in volts); e = base of natural logarithms.

cH_s = cH of the standard buffer solution

pH_s = pH of the standard buffer solution

cH_x = cH of the unknown solution (or tissue)

pH_x = pH of the unknown solution

n = valency of the hydrogen ion (= 1)

F = electric charge carried by an ion (96,540 coulombs)

R = the gas constant

T = the absolute temperature.

Equilibrium is attained immediately, the measurement therefore only occupying a few minutes. It is accurate to 0·01 pH, and only requires 0·5 c.c. of the solution for a determination. The determination of the hydrogen-ion concentration of blood presents no special difficulty. The glass electrode has now been used in very various investigations by Dr. Kerridge and others, with entire satisfaction. One of the earliest results obtained by its use was the demonstration by direct measurement that oxyhæmoglobin is a stronger acid than reduced hæmoglobin.

The arrangement used by Dr. Kerridge is shown in Figs. 36 and 37.

The Indicator Method.—*General Principles.*—Most colour indicators are feeble acids which in the free, and therefore almost undissociated, state have a colour different from that of the salt anion. The anion is present (in an altered form) in solution when there is enough base to combine with the acid, the salt so formed being more or less fully dissociated. Thus neutral red, when present in the state of the free acid,

has a rose-red colour, the free acid, which may be represented as $HR\alpha$, being but feebly dissociated and having that colour. When sufficient alkali is added, the colour changes to yellow, because there is a change in structure of the acid,[1] and the compound formed with base (BR_β) is almost fully dissociated :—

$$BR_\beta = B^{\cdot} + R'_\beta,$$

and the anion R'_β is yellow in colour.

Now the general law of equilibrium between salts of weak acids and the free acids, mentioned on p. 106, applies also to such substances as the indicators, and may be conveniently expressed in the somewhat different form

$$k \cdot \frac{[HR_\alpha]}{[BR_\beta]} = c\mathrm{H},$$

or, in other words, the ratio of the free colour acid to the colour salt (that is, the colour of the mixture) depends on the dissociation constant (which is different for different indicators) and on the hydrogen-ion concentration of the solution in which it is present.

The particular hydrogen-ion concentration at which the indicator most rapidly changes colour depends on the value of k for the indicator in question, and it is obvious that when $c\mathrm{H} = k$ the indicator will be present to the extent of one-half in the form of the free acid and one-half in the form of the salt, so that the colour given by the mixture will be that due to an equal mixture of the two. Indicators can be found with very different ranges, and these are most valuable for the investigation of hydrogen-ion concentration. If to a buffered solution of unknown $c\mathrm{H}$ an appropriate indicator be added, the hydrogen-ion concentration of the solution can be estimated by observation of the colour which the indicator gives with it. Exact determination of the H-ion

[1] It is important to remember that there is a change in the structure of the colour acid molecule as well as in the concentration of the acid anion (see A. W. Stewart). We cannot further discuss this here.

concentration is attained if a mixture of known buffers in known proportions be made which gives the same colour with the same amount of that indicator, because if the nature of the standard buffer solutions is known it is easy to plot mixture curves from which to calculate the H-ion concentration resulting when they are mixed in any proportions.

The list in the table shows some of the more important colour indicators, the ranges of *p*H over which they can be used, and the colours at either extremity of the range.

List of Common Indicators for p*H Determinations*

Indicator.	Range, *p*H.	Acid Colour.	Alkaline Colour.
Methyl violet	0·1— 3·2	Yellow	Violet.
Thymol blue (acid range)	1·2— 2·7	Red	Pale yellow.
Tetrabromphenol-sulphone-phthalein (bromphenol blue)	3·0— 4·6	Yellow	Blue.
Methyl orange	3·2— 4·6	Red	Yellow.
Congo red	3·5— 4·7	Blue	Red.
Methyl red	4·4— 6·0	Red	Yellow.
Neutral red	6·5— 8·4	Red (blue in strong acid solutions).	Yellow.
p-Nitro-phenol	5·0— 7·0	Colourless	Green-yellow
Dibrom-*o*-cresol-sulphone-phthalein (brom cresol purple)	5·2— 6·6	Yellow	Purple.
Phenol red	6·8— 8·4	Yellow	Red.
Brom thymol blue	6·0— 7·6	Yellow	Blue.
o-Cresol-sulphone-phthalein	7·2— 8·8	Reddish	Orange.
α-Naphthol-phthalein	7·6— 8·5	Yellow	Blue.
Thymol blue (thymol sulphone-phthalein) (alkaline range)	8·0— 9·6	Pale yellow	Blue.
o-Cresol-phthalein	8·2— 9·8	Colourless	Red.
Phenol-phthalein	8·3—10·0	Colourless	Red.
Thymol-phthalein	9·3—10·5	Colourless	Blue.
Nitrothymolsulphonephthalein	9·2—11·5	Violet	Yellow.
Alizarin yellow R.	10·2—12·1	Yellow	Orange.
Tropaeolin O	11·1—12·7	Yellow	Orange.

These indicators are useful for a variety of purposes, but for blood reaction neutral red and phenol red cover the required range.

The Indicator Methods for *p*H of Blood.—(1) *The Dialysis Method.*—This method depends on the fact that moderate

dilution of the salts of plasma by dialysis does not alter the reaction. The method here described is Dale and Evans' modification of the method first used by Levy, Rowntree, and Marriott.

A collodion sac holding 2 to 3 c.c. is completely filled with the blood (oxalated), and is allowed to dialyse for twenty

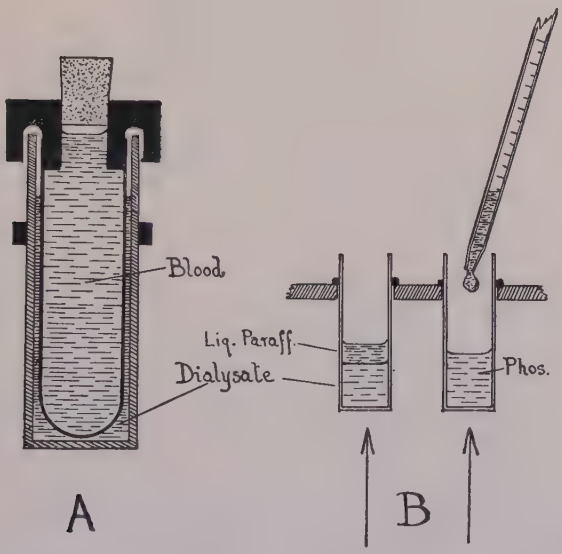

Fig. 38.—Arrangement for dialysis method. A. Dialysis vessel with dialyser filled with blood for the dialysis (full size). B. The arrangement for titration, on the left the dialysate, on the right the phosphate mixture, which is to be titrated till the colour matches that of dialysate (one-half full size).

minutes into 1 c.c. of pure aqueous 0·8 per cent. NaCl solution contained in a flat-bottomed tube, as shown in Fig. 38, A. Loss of carbon dioxide from the blood is prevented by having the dialyser filled to the top and corked; loss from the dialysate is prevented by having the collodion tube made of such a size as almost to fill the outer glass tube, and further by attaching the collodion tube to a vulcanite

cap, as shown in the figure. In this way a dialysate is obtained which contains the free and fixed CO_2 in the same ratio as in the plasma of the dialysed blood. When the period of dialysis is complete, neutral red or phenol red is added in measured amount to the clear colourless dialysate, and in order to hinder loss of CO_2 a layer of colourless liquid paraffin is at once poured on to the dialysate.

The determination of the reaction of the dialysate is carried out by preparing a mixture of phosphates containing the same amount of indicator in a similar flat-bottomed comparator vessel, the mixture being so adjusted as to give the same tint. For the ordinary ranges of blood reaction three solutions are prepared, of the following composition :—

Solution No.	*p*H of Solution.	C.c. m/5 KH_2PO_4.	C.c. n/1 NaOH.	C.c. n/10 NaOH.	C.c. Freshly Boiled Distilled Water.
1	7·5	33·33	—	56	To 100
2	6·5	33·33	—	21·33	To 100
3	10·5	33·33	7·33	—	To 100

By suitable mixtures of two of these three solutions a mixture of any desired *p*H between 6·5 and 10·5 can be prepared. In carrying out the comparative titration, 1 c.c. of solution No. 1 is usually taken, the indicator added in the proper amount, and the colour which is given is compared with that of the blood dialysate. For the comparison the two tubes are placed in a comparator box, so arranged that light is thrown up through the bottoms of the two tubes, which are placed side by side, and observed from above (Fig. 38, B). It will at once be evident whether the phosphate solution is more alkaline or more acid than the dialysate. The reaction of the No. 1 solution is now adjusted by addition from a graduated pipette of measured amounts of either No. 2 or No. 3 until the tint is the same as that of the

dialysate. The amount of the adjusting solution so added is read off, and the *p*H of the phosphate mixture so prepared

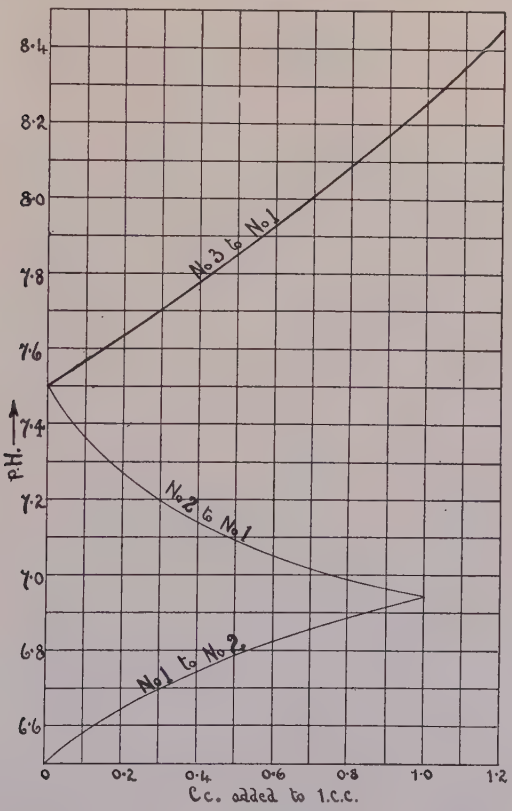

FIG. 39.—Mixing charts for phosphates used in dialysis method. Ordinates = *p*H resulting from mixture shown on abscissæ; abscissæ = volumes in cubic centimetres of—

(*a*) No. 3 added to 1 c.c. No. 1 (upper curve),
(*b*) No. 2 added to 1 c.c. No. 1 (middle curve),
(*c*) No. 1 added to 1 c.c. No. 2 (lowest curve).

is read off by reference to a graph shown in Fig. 39. Thus if it was found necessary to add 0·2 c.c. of No. 3 to the 1 c.c. of No. 1 to produce a match, then the *p*H would be 7·64. For solutions more acid than *p*H 6·94 it is better to start

with 1 c.c. of No. 2 and add No. 1 in measured amount, using the appropriate curve.

(2) *Martin and Lepper's Method.*—This method depends on the fact that direct dilution of blood plasma causes but slight change in its hydrogen-ion concentration.

Only about 20 c.mm. of blood is required. Tubes are made of resistance glass 8 cm. long and 2·5 mm. internal bore, and drawn out to a capillary at each end so as to leave 7·5 cm. from shoulder to shoulder. A tube is filled to within 0·5 cm. of one shoulder with a saline solution containing 0·7 per cent. NaCl and 0·2 per cent. sodium fluoride, to which $\frac{1}{10}$ of its volume of 0·02 per cent. phenol red is added and the *p*H adjusted to 7·5 with M/50 NaOH, containing the same amount of indicator. Blood is drawn by pricking the finger and is allowed to run into the tinted saline so as to fill the remaining 0·5 cm. of the tube, which is then sealed at both ends. After well mixing, the tube is centrifuged and the supernatant tinted plasma-dilution is matched against a set of similar standard tubes 7 cm. long, filled with M/15 phosphate solutions of *p*H varying from 7·3 to 7·6 at intervals of 0·05, to which $\frac{1}{10}$ volume of 0·02 per cent. phenol red has been added. Matching is done by holding the tubes at 45° over a piece of white paper. In order to obtain the *p*H at body temperature it is necessary to deduct 0·2 from the result of the matching at room temperature.

Accuracy of the Colorimetric Methods.—The result obtained by the colorimetric methods (at 20°) for blood equilibrated at 38° is higher by about *p*H 0·2 than that obtained by the electrometric method at 38° C. This effect is due partly to the difference of temperature and partly to dilution, but chiefly to the former. After the application of the proper correction to allow for these factors the results of the two methods very naturally agree. Various observers find that there is exact agreement between the colorimetric and electrometric methods, when both are carried out at

the same temperature. Bayliss, Kerridge and Verney have checked the indicator method against the hydrogen and glass electrodes, and obtained identical results with all three methods. For blood in equilibrium at 38°, and dialysed or diluted at 20°, deduction of 0·2 from the observed *p*H is necessary.

The CO_2 Reaction Curve of Blood and of other Solutions.—When the *p*H of a liquid in equilibrium with different pressures of carbon dioxide is determined, the results may be plotted as a graph relating CO_2 pressures to *p*H (or *c*H)

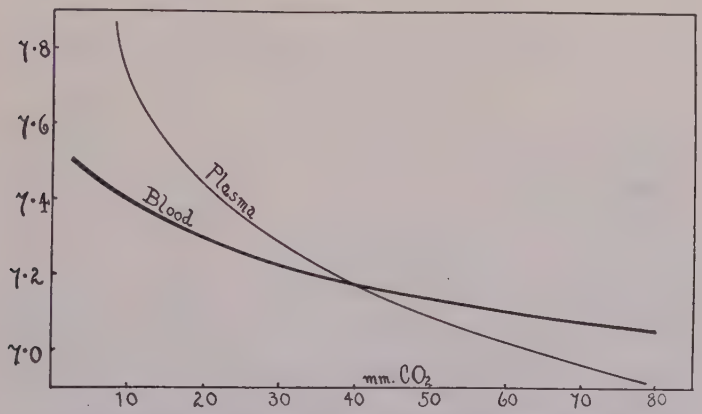

FIG. 40.—CO_2 reaction curves of blood and of plasma separated from it.

and this has been called the CO_2 reaction curve. The equilibration is carried out precisely as described on p. 82. If a perfectly buffered solution could be obtained, it would have the same reaction at all CO_2 pressures; the curve would be a straight horizontal line. The more perfectly any solution is buffered, the more nearly will it approach to this line, and the less perfectly it is buffered, the steeper will be the slope of the CO_2 reaction curve.

A glance at Fig. 40, which shows the CO_2 reaction curves for blood and for separated plasma, tells us at once that blood is much more perfectly buffered than is separated plasma.

By the reaction of " blood " as ordinarily expressed we mean the reaction of the true plasma of the blood. There is thus a great difference between the buffering power of separated plasma, which relies only on its own buffers, and that of the true plasma, which, as we have learned (p. 87), has the resources of the red corpuscles to draw upon as well. In Fig. 40 it will be seen that the curve for plasma crosses that for blood at about 39 mm. CO_2; at this pressure, therefore, the separated and true plasmas are identical, or, in other

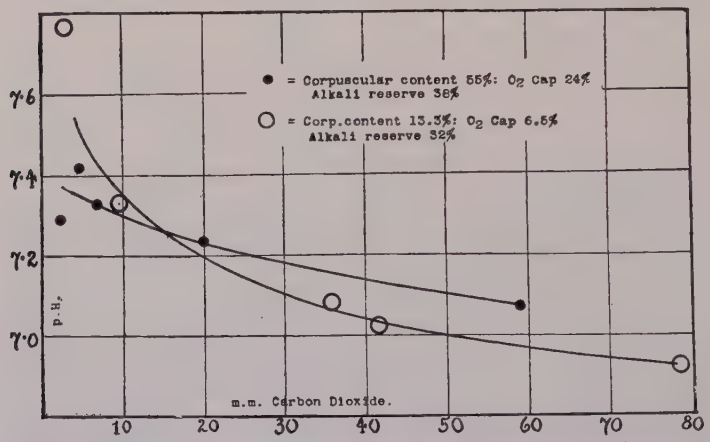

FIG. 41.—Effect of corpuscular content on the buffering of the blood. (From *Journal of Physiology*, 1921, 55.)

words, the separated plasma was drawn from the blood at a CO_2 pressure of 39 mm. Hg. At all pressures less than this the separated plasma is more alkaline than the blood, while at pressures greater than 39 mm. the plasma is more acid than the blood; *i.e.*, the ratio $CO_2/BHCO_3$ is in the first case smaller, and in the second case greater, in plasma than in blood (or true plasma). Such a result is exactly in accord with what we have already seen to happen to the bicarbonate content of separated and true plasmas at different CO_2 pressures, as shown on the CO_2 dissociation curves. When CO_2 is added to blood, we have seen that the bicarbonate

content of the true plasma is rapidly increased, and this fully accounts for the superior buffering of blood as compared with separated plasma, in which such rapid increases of bicarbonate content do not occur. This effect of the corpuscles, which respond to addition of CO_2 by producing more bicarbonate in the true plasma, is called the *secondary buffering* of blood, to distinguish it from the type of buffering seen in separated plasma, which is spoken of as *primary buffering.*

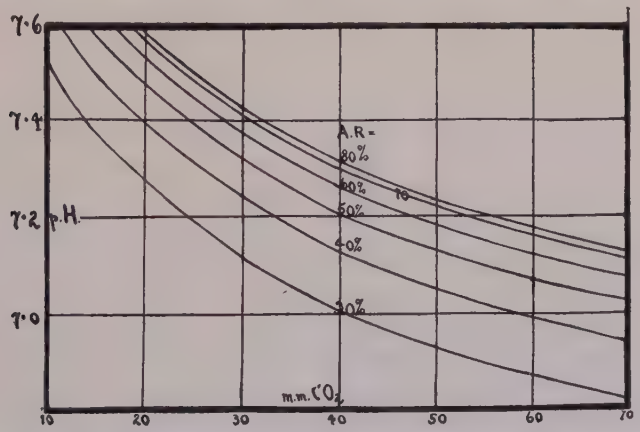

FIG. 42.—CO_2 reaction curves for plasmas of different bicarbonate contents. The figures above the curves show the alkali reserve of the plasma in volumes of CO_2 per cent. (*Journal of Physiology*, 1921, 55.)

It would be expected that the perfection of buffering of any blood would largely depend on its corpuscular (or hæmoglobin) content. That this is so may be seen from inspection of Fig. 41, in which the CO_2 reaction curves of two samples of the same blood of different corpuscular contents are compared. It is obvious that the one with the higher corpuscle content has much the better buffering.

As regards the primary buffering of the plasma, this is almost entirely due to the bicarbonate which it contains, and Fig. 42 shows that as the primary buffering of the plasma is increased, as it can be by addition of sodium bicarbonate,

the only effect is to raise the curve to a higher level, which means that its relative, but not its absolute, buffering power is increased, all the curves being approximately parallel.

The Reaction of Blood in the Body.—Arterial blood, recently shed, and examined at body temperature, and without alteration of its gases, has a reaction slightly on the alkaline side of neutrality, viz. about *p*H 7·35 to 7·4 at the normal body temperature. This is therefore taken as the normal *p*H of arterial blood; it is raised by cooling, *e.g.*, cooling to 20° raises the *p*H by about 0·15. The reaction is kept very nearly constant, even under the most variable conditions, in health, and it is not certain that life could be maintained for long if it underwent any great alteration.

According to recent work by Havard and Tookey Kerridge, the reaction of blood in the body is slightly more alkaline than was formerly supposed. They found that when the reaction of fresh blood was taken, which can be done very rapidly by using a glass electrode, the *p*H shows a fall of about 0·05 in about five minutes at 38°, less rapidly at lower temperatures, *e.g.* at 20° not till about two hours after shedding. This sudden change occurs in whole or laked blood, but not in plasma; it is unaffected by anticoagulants, fluorides, thymol, heptyl alcohol, urethane or cyanides, and its cause is unexplained.

Very frequently in pathological states it is found that the bicarbonate content (alkali reserve) of the plasma is lowered owing to generation of fixed acids in the body. This state is often called "*acidosis*." More often than not the respiration is adjusted so as to keep the acid-base ratio unaltered, so that the reaction is unchanged: this may be called a "*compensated acidosis*." There are, nevertheless, definite differences in H-ion concentration under circumstances in which the ratio of free to fixed carbon dioxide is altered. The most important of these is during muscular exercise. When this is violent there is a considerable accumulation of lactic acid in the blood, which displaces some of the fixed

CO_2 by decomposing the bicarbonate. Although this tendency is to some extent countered by the ionic interchange, by the simultaneous deoxygenation of the blood, and by increase of respiratory ventilation which removes CO_2, and so tends to restore the acid-base ratio, for a time the destruction of bicarbonate often assumes the upper hand, with the result that the blood becomes less alkaline than normal. In diseases in which large amounts of fixed acid are produced, *e.g.*, in diabetes, the destruction of bicarbonate may be so continuous and extensive that it cannot be counteracted by increase of respiration, and a definite condition of *acidæmia* is established. J. B. S. Haldane has shown that after ingestion of ammonium chloride in large amounts there is a state of acidosis, and, no doubt, often of acidæmia ; this is explained by the conversion of the ammonium radicle into urea, leaving the acid radicle behind in the body.

Changes in the direction of an increased alkalinity of the blood are most clearly present as a result of excessive pulmonary ventilation, especially if this is unaccompanied by vigorous muscular exertion. This condition may be called *alkalæmia*, and is obviously due to the too rapid removal of carbon dioxide from the blood, so disturbing the acid-base balance on which the reaction depends. This state of affairs is found in individuals rapidly brought into atmospheres where the oxygen tension is low, as at high altitudes. The breathing is exaggerated, CO_2 is removed, and there follows a state of alkalæmia. But this is soon rectified by the excretion of more base by the kidneys, the urine becoming alkaline in reaction, and the bicarbonate content of the blood falling proportionally, so that the alkalæmia is only temporary. The hyperpnœa caused by a rise of body temperature has been shown by Bazett and J. B. S. Haldane to produce similar effects as regards the alkalinity of the urine.

The Change from Arterial to Venous Blood.—*The Blood Diagram.*—Up to the present we have only considered the

H-ion concentration of fully oxygenated blood. It is evident that the considerations dealt with in the last chapter in connection with the carbon dioxide carriage of the blood must also have important applications as regards its hydrogen-ion concentration. For, in passing from the arterial to the venous state, not only is the carbon dioxide pressure raised, but the oxygen content of the blood is lowered, and we have seen that this enables the blood to carry more carbon dioxide in its true plasma (as $NaHCO_3$) than it would be able to do if there had not been this accompanying reduction of the oxyhæmoglobin. We should expect, therefore, since for a given increment of carbon dioxide pressure (*i.e.*, free CO_2) there is a greater compensatory increase in the fixed CO_2, that the change in H-ion concentration in passing from the arterial to the venous state would be much smaller than would be expected from inspection of the CO_2 reaction curve of fully oxygenated blood. This is found to be what actually occurs, and may be illustrated by means of the blood diagram introduced by Henderson and Haggard, and which we will now briefly describe.

Knowing that the reaction of the blood depends on the ratio of CO_2 free to CO_2 fixed, it is obvious that, if this ratio remains the same, the reaction will be unaltered. We can easily calculate the *p*H for a given ratio, and from this draw a line corresponding to this ratio on the CO_2 dissociation curve co-ordinates. If a number of these lines (*p*H isopleths), corresponding to certain *p*H's, be so drawn, we have a means of estimating the *p*H of the blood at any parts of the dissociation curve. Such a blood diagram is shown in Fig. 43.[1]

When we plot out a CO_2 dissociation curve on such a diagram, the point in the curve which falls on any of the *p*H isopleths will be the point at which the blood has the reaction corresponding to that isopleth. In the figure shown, for

[1] Full details of how to calculate the position of these *p*H isopleths will be found in Van Slyke's article. It may be stated here that the lines for a blood diagram can be constructed as follows: Find the point where the abscissa (total CO_2 content) of 70 volumes per cent. crosses the line for a

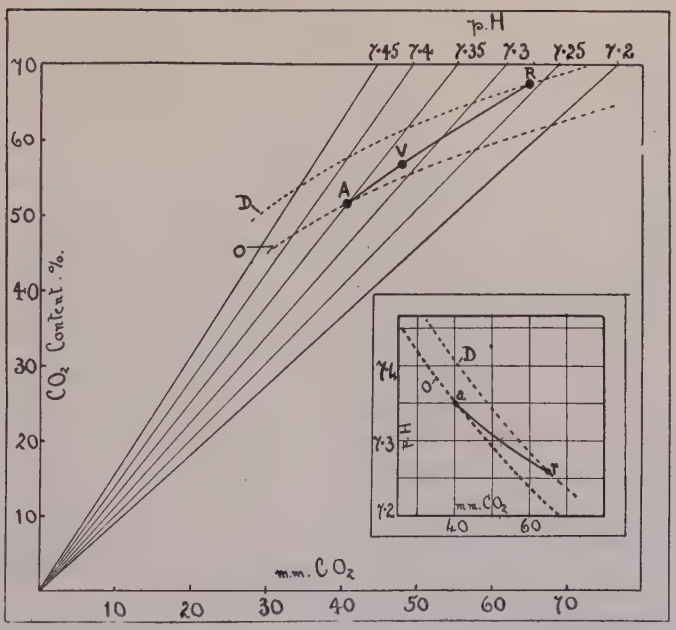

FIG. 43.—*Main figure:* Part of CO_2 dissociation curve copied from Christiansen, Douglas and Haldane's paper, with addition of *p*H isopleths, constructed as described in text. Dotted curve O = oxygenated blood; D = reduced blood; curve AVR = physiological CO_2 dissociation curve, in which CO_2 addition is accompanied by oxygen removal.

Small curve inset: CO_2 reaction curves drawn from the data of the main curve. The physiological CO_2 reaction curve (*a*—*r*) shows better buffering than the other two.

instance, fully oxygenated blood at a CO_2 pressure of 40·5 mm. has a *p*H of 7·35. Fully reduced blood does not reach this *p*H until the CO_2 pressure has been raised to about 48 mm.

pressure of 76·6 mm. CO_2. Join this with the zero, and the oblique line thus obtained is the isopleth for *p*H 7·20.

Similarly, join to the zero the following points on the horizontal line of total CO_2 = 70 volumes per cent.: —

CO_2 pressure,	68·9 mm.	=	line for *p*H,	7·25.
,,	61·8 ,,	=	,, ,,	7·30.
,,	55·4 ,,	=	,, ,,	7·35.
,,	49·4 ,,	=	,, ,,	7·40.
,,	44·5 ,,	=	,, ,,	7·45.

Considering the physiological CO_2 curve AVR, which is that discussed on p. 96, we see that in passing from arterial blood containing 51·5 volumes of CO_2 per cent. to venous blood containing 57 volumes per cent. there is a change in reaction from *p*H 7·35 to about 7·32, *i.e.*, of only *p*H 0·03. This actually is the order of the difference which is found by direct examination of the two kinds of blood. Were it not for the effect of the simultaneous reduction of oxyhæmoglobin in improving the CO_2 fixation, it can be seen from this diagram that the blood when it contained 57 volumes of CO_2 per cent. would have had a CO_2 pressure of 53·5 mm., and a *p*H of 7·27, a fall of 0·08 as compared with the arterial blood.

These facts are illustrated by the small diagram inset in Fig. 43, which shows the CO_2 reaction curves deduced from the curves in the larger figure. The physiological curve, in which carbon dioxide addition is accompanied by oxygen withdrawal, shows that it is obviously better buffered than either of the other two.

The Reaction of the Corpuscles.—The general principles governing the reaction of the contents of the corpuscles are the same as those which operate in the plasma or elsewhere ; that is to say, we have inside the corpuscles an equilibrium between certain weak acids (Hb, HbO_2, KH_2PO_4, H_2CO_3) and their salts. There must also be a state of equilibrium between the contents of the corpuscles and the substances in the plasma. Owing to the fact that the envelope of the red cell is only partially permeable to the constituents of the cell on the one hand and those of the plasma on the other hand, this equilibrium between corpuscular contents and plasma is not so simple as would at first sight appear, for, in addition to the possibilities of the existence of an equilibrium on the basis of the law of mass action, we have to take into account the " Donnan membrane equilibrium " (see p. 93). Suppose that the potassium salt of hæmoglobin ionises in solution to give $K^{\cdot}$ ions and Hb' ions. When carbon dioxide

is added to the blood, it passes through the corpuscular membrane, and reacts with hæmoglobin to form the acid HHb and $KHCO_3$.

The free acid of hæmoglobin (HHb) will be dissociated into its ions H· and Hb′, but since the Hb ion is unable to diffuse out, it restrains the H ion by electrostatic attraction from wandering through the membrane. Hence the hydrogen ion inside the corpuscle keeps at a higher level than that in the plasma. Another consequence of the membrane equilibrium is that the red cells carry a negative charge with reference to the plasma, *i.e.*, the cells move towards the anode in an electric field.[1] The ionic interchange referred to in the last chapter is due to the peculiar partial permeability of the envelope of the red cell.

By direct experiment it has been shown that hæmolysed corpuscles have at the same CO_2 pressures a higher hydrogen-ion concentration than plasma, and this difference is found to be of about the same order as that calculated from the Donnan ratio, viz., about *p*H 0·12. Further, the value of K, the dissociation constant for oxyhæmoglobin,[2] varies in blood inversely as the *c*H of the plasma, and this has been interpreted by A. V. Hill as indicating that the dissociation of oxyhæmoglobin depends on the reaction inside the corpuscles.

The Nomogram.—We know that the saturation of the blood with oxygen is a function of the oxygen pressure which may be expressed by the oxygen dissociation curve, and that the amount of carbon dioxide held in the blood

[1] This should be so only so long as the Hb functions as an acid. When the isoelectric point is passed and Hb functions as a base, combining with CO_2, as explained on p. 98, then the positively charged Hb ion would electrically retain the CO_3' ion, with the result that the CO_3'-ion concentration would be greater, and the H′-ion concentration less, inside the corpuscle than outside (H. C. Wilson); the electric charge would also be reversed. Such a condition would not occur in the body, however.

[2] In Hills' equation:—$y = 100\,\frac{Kx^n}{1 + Kx^n}$ where y = % saturation of hæmoglobin with oxygen, x = the oxygen pressure, n = a constant of about 2·5, and K, the dissociation constant.

varies with the carbon dioxide pressure, as shown by the CO_2 dissociation curve. It has also been learned that there is a relation between the two curves, for addition of carbon dioxide affects the oxygen dissociation curve in a definite manner, and conversely the addition of oxygen has a definitely predictable effect on the carbon dioxide dissociation curve.

We similarly know that alteration of the carbon dioxide pressure alters the hydrogen-ion concentration, and also the chloride concentration of the plasma. Finally, it has been demonstrated that alteration of hydrogen-ion concentration affects the oxygen dissociation.

A little reflection makes it clear that all these properties of the blood must be related to one another. L. J. Henderson has shown that, in fact, blood can conveniently be regarded as a complex physico-chemical system in which at least seven important variables are concerned. These variables are—

(1) The free oxygen (expressed by oxygen partial pressure).

(2) The combined oxygen (oxygen saturation of hæmoglobin).

(3) The free carbon dioxide (CO_2 partial pressure).

(4) The combined carbon dioxide (fixed CO_2).

(5) The hydrogen-ion concentration of the plasma.

(6) The chloride concentration of the plasma.

(7) The water distribution between corpuscles and plasma.

Now the relation between these and other variables is such that, if *any two* of them are arbitrarily fixed at definite values, the remainder will thereby be fixed according to definite laws of chemical equilibrium. For instance, we know that if the partial pressures of oxygen and of CO_2 are given particular values, the hydrogen-ion concentration, the oxygen saturation, CO_2 content, and chloride content will acquire certain fixed values. Conversely, if we take blood in a state of equilibrium, all the variables will have a certain value ; if we alter any one of them, there will result

compensatory changes in the others, a new state of equilibrium being attained.

L. J. Henderson has shown that the mutual relationships of these variables can be expressed in a chart drawn on superposed co-ordinates, and called a *Cartesian nomogram*. From

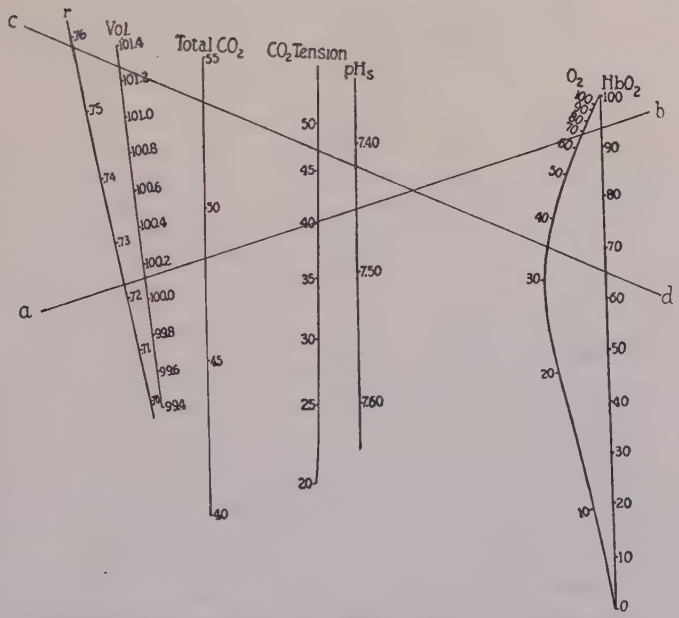

Fig. 44.—Alignment chart for blood. (From L. J. Henderson *Lectures on Certain Aspects of Biochemistry.*)

this diagram it is possible to construct an alignment chart (or d'Ocagne nomogram) of the type shown in Fig. 44. In using the nomogram it is only necessary to join any two known points by a straight line, which if produced will then cut the other points and give the remaining data for that particular blood. Thus the line *ab* might represent the conditions in arterial blood, and the line *cd* those in a venous blood. Various other data are expressed in, or readily deducible from, this nomogram, which may be said to repre-

sent in graphic form the law of the blood. The line marked r represents the "Donnan ratio," and expresses the equilibrium between the serum and corpuscles, *i.e.* :—

$$r = \frac{[BHCO_3]_c}{[BHCO_3]_s} = \frac{[HCO_3']_c}{[HCO_3']_s} = \frac{[Cl']_c}{[Cl']_s} = \frac{[H\cdot]_s}{[H\cdot]_c},$$

while the line *Vol.* represents the volume of the corpuscles; as a percentage of their volume at $O_2 = 80$ mm. and $CO_2 = 39$ mm. The line pH_s shows the pH of serum (or plasma).

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CHAPTER VI

THE CHEMISTRY OF MUSCULAR CONTRACTION

Tissue Respiration

The cells of all tissues of the higher animals continually use up oxygen, if it is available, throughout their lives, and the energy of the various life processes is derived chiefly, if not entirely, from oxidative reactions carried out by the living cells. This observation led many to believe that life could only be kept up in a tissue so long as it was actually carrying out oxidative changes, and that when these ceased it was dead. When a tissue, such as muscle, performed work, it utilised oxygen at an increased rate, but even when at rest it still used some oxygen and continued to produce carbon dioxide.

A difficulty was encountered in the fact that most tissues could support total oxygen lack for variable lengths of time, and then, if the deprivation of oxygen were not too prolonged, could resume their respiratory processes as soon as it was readmitted. When oxygen was withheld in this way, it was noticed that the liberation of carbon dioxide by the tissue still continued for a long time, and further, that on renewal of the oxygen supply the tissue at first used much more oxygen in a given time than during a similar period before the asphyxia. The excess oxygen used in recovery from asphyxia over and above the resting requirement of the tissue was shown to be equal to the oxygen usage which was omitted during the anaerobic period.

The earlier physiologists believed that the tissues contained a store of available oxygen on which they could draw during periods in which oxygen access was withheld. This

was called *intramolecular oxygen*, and at one time the theory of intramolecular oxygen, now discredited, was in general favour.

According to the theory of intramolecular oxygen, the tissue was able, by drawing upon its store of oxygen, to carry out oxidations in absence of free oxygen, and so for long periods to produce carbon dioxide. Ultimately the intramolecular store of oxygen was supposed to become exhausted, and the tissues then either died or showed suspense of function. When oxygen was readmitted, the tissues resumed their respiration, and also took in a fresh supply of oxygen to make up the depleted intramolecular store; hence the increased oxygen usage after asphyxia.

At the present day these facts are explained quite differently. In the absence of oxygen there is an accumulation of lactic acid in the tissues; this will expel its equivalent of carbon dioxide from combination as bicarbonates in the tissues, which explains the continued production of carbon dioxide during anaerobic periods. When oxygen is again presented to the tissues, the accumulated lactic acid is removed by an oxidative process, and so we get the accelerated oxygen usage together with the recovery of function.

These considerations show that contemporary utilisation of molecular oxygen is not an essential accompaniment of the life processes, and that life can proceed for short periods without it; the essential thing appears to be the production of metabolites, of which lactic acid, though no doubt not the only one, may be taken as a type. Oxygen acts by removing the lactic acid, which in its absence accumulates and stops the working of the machine. These phenomena will be more fully discussed later; the point to be made here is that oxygen is not the prime mover in the life processes, but an accessory which enables them to continue. Its utilisation provides energy which, so to speak, winds up the machine for a new run.

The Determination of the Oxygen Usage of Tissues.—For the

investigation of the oxygen utilisation of isolated tissues much use has been made of the *microrespirometer*, the principle of which is illustrated by Fig. 45. The two small flasks A and B are of equal size, and are connected each with one limb of a fine-bore manometer filled with kerosene and provided with a millimetre scale. A Barcroft's blood-gas apparatus, fitted with bottles of suitable shape and with a finer bore manometer than that usually provided, serves well for the purpose. On the bottom of each of the flasks is a shallow receptacle (*a*, *b*) in which is placed a few drops of potash solution; the tissue, cut up or minced, is floated in Ringer's or other suitable solution in the bottom of one flask, the other containing Ringer's solution only. The bottles are placed in a thermostat and the air replaced with oxygen. The meniscus of the manometer is levelled, and the whole apparatus is continuously agitated by a suitable shaking apparatus, by which it is moved in a horizontal direction to and fro. At intervals the reading of the meniscus in each limb of the manometer is taken. The level of the kerosene ascends in the manometer on the side connected with the bottle containing the tissue, and falls on the other side. The carbon dioxide produced by the tissue is absorbed by the potash, so that the movement of the kerosene is entirely due to the oxygen absorbed, the volume of which can be calculated if the constant of the apparatus is known. Thus the oxygen utilisation of the tissue under given conditions can be found over any required period of time.

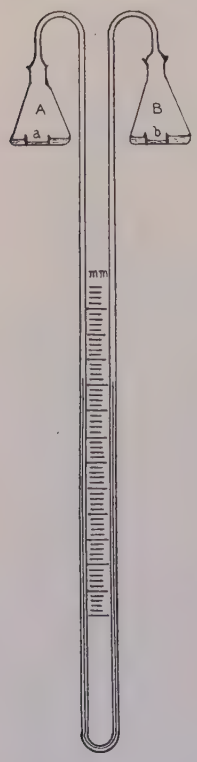


FIG. 45.

If a parallel experiment is made with omission of the potash from the receptacles, the change of volume is due to the difference between the oxygen used and (allowing for its solubility) the carbon dioxide formed, and from this, if the oxygen used is known, the respiratory quotient can be calculated.

Lactic Acid Formation and Removal

Present-day views of the chemistry of muscular contraction have their origin in the investigations of Fletcher on the respiration of muscle and of Fletcher and Hopkins in

1907 on the lactic acid content of muscle. It had long been known that lactic acid appeared in a muscle stimulated to the point of fatigue, and that means existed in the body for the removal of this lactic acid, but the site and mode of its removal had not hitherto been traced.

Fletcher and Hopkins studied the lactic acid content of frog's muscle under a variety of conditions; the lactic acid content of fresh resting muscle was found to be low provided that special precautions had been taken to avoid stimulating and damaging the muscle in the course of the preparations prior to its extraction. The resting minimum of lactic acid was found to be somewhere about 0·015 per cent., and it may be supposed that had the effects of incidental stimulation been eliminated, the lactic acid content of the resting muscle would have been negligibly small.

When the muscle had previously been stimulated to exhaustion, the lactic acid had reached a value of at least 0·16 per cent., which fatigue maximum was not further increased, however long the muscle was subjected to stimulation.

When the fatigued muscle is placed in oxygen, the lactic acid will in some hours fall even to the resting value if the conditions for access of oxygen to the muscle fibres are adequate. The removal of the lactic acid, therefore, can take place in the muscle itself. Together with this reduction in the lactic acid, the muscle regains its excitability, and if it is then subjected to a further exhaustive stimulation, there is once more a development of lactic acid up to the fatigue maximum. In this way the muscle can be fatigued and restored as long as it remains alive.

The inference from these experiments was that the lactic acid was derived from the break-down of some precursor when the muscle entered into contraction, and it seemed not unlikely that the acid played a definite part in the causation of the contraction itself. When oxygen was admitted to the exhausted muscle, the evidence seemed to show that

the lactic acid was reinstated as precursor again, thus rendering the muscle capable of a fresh performance on excitation.

It was further shown that when a muscle was placed out of contact with oxygen its lactic acid content rose steadily until the muscle passed into rigor, which occurred much earlier under these conditions than it did when the muscle was placed in air, or, better, in oxygen. Other experiments showed that a high lactic acid content was reached when the muscle was sent into rigor by any means. The inference lay near that under ordinary circumstances a muscle passed into a state of rigor when the lactic acid had accumulated in it to a particular concentration, thus lending a certain support to the older view that contraction could be regarded as an incomplete and reversible form of the same processes which constituted rigor.

The Chemical Changes in Resting Muscle

If a thin muscle be placed in oxygen in a microrespirometer, its resting oxygen usage is found to be quite small. Meyerhof, for instance, found it to be in one experiment about 30 c.mm. oxygen per gram per hour at 22° C., and in another instance 11 c.mm. at 14°. A thin muscle was used in order to ensure that oxygen readily penetrated to the interior of the tissue.

Estimation of the lactic acid of the resting muscle showed this to be of the order of 0·015 per cent.

For estimations of lactic acid, the tissue is killed by cold alcohol or liquid air, minced, and the lactic acid appropriately extracted, *e.g.* by alcohol. The tissue residue may be used for the estimation of glycogen by a micro-modification of Pflüger's method. Or the lactic acid may be extracted direct from the muscle by grinding with trichloracetic acid. The lactic acid in either extract, after suitable purification, is oxidised with acid permanganate, whereby the lactic acid is converted into acetaldehyde ; this is driven off and trapped in sodium bisulphite, and the bisulphite thus bound estimated by titration with n/100 iodine.

Determination of the glycogen content of the resting muscle at the beginning and end of a period of survival in oxygen or other gas enables a comparison of the carbohydrate and oxygen usage to be made. The following is an example from Meyerhof's papers :—

Per Gram Muscle in Forty-five Hours. (Meyerhof.)

Condition.	c.mm. O_2 used.	mg. Carbohydrate (as glucose). Before.	After.	mg. Carbohydrate, difference.	mg. Lactic Acid formed.
In O_2 (14°)	980	11·11	9·88	— 1·23 (= 1·30 corr.)	—
In N_2 (14°)	0·0	9·40	5·85	— 3·55 (= 3·75 corr.)	+ 2·62 mg.

For a loss of 1·30 mg. of glycogen (expressed as glucose) there has been an oxygen utilisation of 980 c.mm. Now, from the equation

$$C_6H_{10}O_5 + 6\ O_2 = 6\ CO_2 + 5\ H_2O$$

it can be calculated that 162 mg. glycogen (= 180 mg. glucose) use up 133 c.c. oxygen, and hence that 1·3 mg. of glucose would utilise about 960 c.mm. oxygen,which is in very good agreement with what was actually found. The net result then, in resting muscle is a combustion of glycogen, with utilisation of oxygen and production of carbon dioxide in calculated amounts.

When the muscle was placed in nitrogen or in oxygen-free Ringer's solution (anaerobiosis), it was found, as shown in the table, that the amount of lactic acid which accumulated was of about the same order as the glycogen which was lost. When certain disturbing effects are allowed for, the whole of the glycogen disappearing is recoverable as lactic acid.

The lactic acid continues to increase during the stay in

nitrogen, until a definite maximum is reached, shortly after which the muscle dies if still kept without oxygen ; but if returned to oxygen, the muscle recovers, and this return of function is accompanied by three definite chemical phenomena : (1) disappearance of the lactic acid, (2) increase of glycogen, and (3) utilisation of oxygen at a rate greater than that previous to the period of anaerobiosis. This increased oxygen usage (called " excess oxygen ") is at first high ; it subsides, at first quickly, then more slowly, to the original basal level, which is only reached after many hours. As soon as the oxygen usage has returned to the resting level, but not before, the lactic acid is also found to have the low value which characterises the resting tissue.

If lactate or pyruvate is added to the Ringer's solution it also disappears by conversion to glycogen ; it is stated that the natural *d*-lactic acid can be synthesised to carbohydrate much more readily than the *l*-isomer.

The " excess CO_2 " is found to be equal to the excess oxygen, so that the respiratory quotient of the recovery process is unity ; that is to say, it agrees with the belief that the oxygen which is utilised in recovery burns up lactic acid or a carbohydrate.

But when we compare the excess oxygen usage in recovery with the amount of lactic acid which has disappeared, it at once becomes clear that the whole of the lactic acid cannot have been removed by oxidation, for, according to the equation

$$\underset{(90\ \text{grm.})}{C_3H_6O_3} + 3\ O_2 = 3\ CO_2 + 3\ H_2O \qquad \underset{(67\ \text{l.})}{}$$

each milligram of lactic acid removed should require 744 c.mm. of oxygen for its oxidation, whereas as an average in the experiment of Table p. 141, only 237 c.mm. oxygen is used for each milligram lactic acid lost, *i.e.*, only about one-third of the calculated amount. There is reason to believe that under normal conditions a usage of only 149 c.mm. of oxygen per milligram of lactic acid lost is an average figure,

Oxygen and Lactic Acid in Recovery from Anaerobiosis.
(Modified from Meyerhof.)

Duration of Anaerobiosis.	Duration of Recovery.	Excess O_2 used per gram during Recovery, c.mm.	mg. Lactic Acid lost in Recovery per gram.	Oxygen needed to oxidise all Lactic Acid lost in Recovery, c.mm.
6 hours	18·5 hours	135	0·43	320
17 ,,	10·75 ,,	113	0·60	446
19 ,,	8·75 ,,	129	0·56	417

that is, about one-fifth of the amount which would be needed for complete oxidation of the lactic acid instead of one-third, as in the experiment given above. The fraction varies with the condition of the muscle, becoming larger the less efficient its operation. A. V. Hill finds that in fresh muscles subjected to short periods of stimulation the recovery efficiency is one-sixth. This would mean that of the lactic acid set free during contraction one-sixth is oxidised to CO_2 and water, and five-sixths is restored to glycogen.

The fate of the lactic acid which is not oxidised becomes clear when the glycogen content of the muscle is followed during recovery, for there is an increase in the glycogen content of the muscle which is equivalent to the amount of lactic acid removed, but not oxidised. We can thus write the following equations for the anaerobic and recovery stages concerned in the formation and subsequent removal of 1 gram of lactic acid, assuming recovery efficiency of one-fifth.

(1) $\underset{\text{Glycogen.}}{C_6H_{10}O_5} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} = 2\ \underset{\text{Lactic acid.}}{C_3H_6O_3}$

$162 \text{ grams} \longrightarrow 180 \text{ grams}$

$\therefore 0{\cdot}9 \text{ gram} \longrightarrow 1 \text{ gram}$ (O_2 usage missed in anaerobic period = 149 c.c.)

} Anaerobic period.

(2) 1 gram lactic acid $\longrightarrow$ 0·2 gram oxidised

$\underset{(0{\cdot}2\text{ gram})}{C_3H_6O_3} + 3\ O_2 = 3\ CO_2 + 3\ H_2O$
$\qquad\qquad (149\text{ c.c.}) \quad (149\text{ c.c.})$

$\longrightarrow$ 0·8 gram converted to glycogen (0·72 gram)

$2\ \underset{(0{\cdot}8\text{ gram})}{C_3H_6O_3} = \underset{(0{\cdot}72\text{ gram})}{C_6H_{10}O_5} + H_2O$

} Recovery period.

The excess oxygen used during the recovery will be equal to that omitted (the " oxygen debt ") during the anaerobic period, namely 149 c.c. per gram of lactic acid accumulated. The net result of the whole cycle of anaerobiosis and recovery will be that 0·9 − 0·72 = 0·18 gram of glycogen has been lost, just as though it had been burned directly to CO_2 and water :—

$$\underset{(0\cdot18\text{ gram})}{C_6H_{10}O_5} + \underset{(149\text{ c.c.})}{6\ O_2} = \underset{(149\text{ c.c.})}{6\ CO_2} + 5\ H_2O$$

During a similar period of existence in presence of oxygen it would have been found that the same amounts of glycogen and oxygen would have been used, and the same amount of carbon dioxide formed. But there would have been no accumulation of lactic acid. Thus in one of Meyerhof's experiments a frog's muscle kept in oxygen lost 1·3 mg. of carbohydrate (as glucose) per gram of muscle in forty-five and a half hours and used 1·40 mg. of oxygen, which would be the amount necessary to oxidise 1·31 mg. of glucose. It seems clear, then, that by separating the time into an anaerobic period and a recovery period we have gained an insight into the chemical processes which are going on in the resting muscle under normal conditions. It is easy to believe that under normal conditions the lactic acid is being produced in the tissue at the same rate as during anaerobiosis, but fails to accumulate, not because it is all of it oxidised away, but because the recovery process which we have detected during restoration from anaerobiosis is going on continuously. We may say, then, that 149 c.c. of oxygen, by oxidising 0·2 gram of lactic acid, has prevented the accumulation of 1 gram of that substance in the muscle tissue.

The view given above is the one which has hitherto been prevalent, but there are indications that it may need to be modified. Examination of the carbohydrate content of the muscle after restitution shows, as already explained, that an amount of carbohydrate corresponding to the oxygen utilised has

disappeared, and it seems more in keeping with what happens in fermentation processes to suppose that this carbohydrate has been oxidised by another route in which lactic acid is not a stage. Our changes could thus be written equally well as follows :—

0·9 gram glycogen ⟶ 1 gram lactic acid (anaerobic)
1 gram lactic acid ⟶ 0·9 gram glycogen
0·18 gram glycogen (or equivalent of other carbohydrate) ⟶ $CO_2 + H_2O$ } aerobic.

the energy requisite for conversion of all the lactic acid to carbohydrate being derived by combustion of some of the existing (or resynthesised) carbohydrate. Whether lactic acid accumulates or disappears will depend upon the relative velocities of these two independent processes, the one anaerobic, the other aerobic.

Chemical Changes in Muscle during Activity

When muscle is stimulated its chemical changes are accelerated, though qualitatively the same as during rest. For this reason most of the investigations on the chemical changes in muscle have been made by comparing the resting with the stimulated tissue.

If the muscle is stimulated in nitrogen or moistened with Ringer's solution containing a trace of cyanide to reduce oxidative processes, lactic acid very quickly accumulates, and the " fatigue maximum," is soon reached. If the muscle is a thin one, *e.g.*, the frog's sartorius, and it be suspended in oxygen-free Ringer's solution, then the lactic acid diffuses away and fatigue does not set in so soon, in fact, not until the glycogen has been exhausted. Immersion in Ringer-Locke fluid (0·1 per cent. glucose), by enabling the glycogen to be replenished, still further augments the maximum performance in absence of oxygen (Hill and Kupalov).

When oxygen is readmitted to a muscle which has been brought to fatigue in nitrogen, the oxygen usage is raised above the former resting value, while the " oxygen debt " contracted during the anaerobic period of stimulation is being wiped off ; this corresponds with removal of lactic acid and recovery of excitability of the muscle. The recovery process in a thick excised muscle may take as long

as twenty-four hours. In an atmosphere of oxygen the effect of stimulating the muscle is to raise its oxygen usage to ten or fifteen times the resting value ; if the muscle were thin enough to allow of access of oxygen to all its parts, lactic acid would not accumulate, as a state of balance would be reached, the oxidation-restitution process keeping pace with the production of lactic acid. In experiments with excised muscles these conditions were shown by Hill and Kupalov to be realised when frog's sartorii were suspended in oxygenated Ringer's solution and stimulated twenty to thirty times a minute. Such muscle could give more than 10,000 twitches and oxidise more than $1\frac{1}{4}$ per cent. of carbohydrate. In the living body the richness of the capillary supply (see Chapter III.) gives very favourable conditions for recovery, so that the rate of disappearance of lactic acid is about eight times as fast in a muscle *in situ* as in an excised muscle. A further factor is the possibility of lactic acid diffusing away by the blood stream as soon as the rate of production begins to exceed the recovery velocity. Mrs. G. P. Eggleton and the writer have found that this regularly happens under experimental conditions in mammals ; within half a minute after a short tetanic contraction, the venous blood coming away from the muscle contains lactic acid at a concentration almost equal to that in the muscle, and these experiments seem to suggest that under normal conditions in mammals much of the lactic acid produced in powerful contractions passes out of the muscle into the blood stream.

Experiments on the heat production in contracting and recovering muscles have given information which is quantitatively more precise than that obtained from chemical investigations, because the method is so delicate that much smaller changes can be studied. Thus a single twitch generates sufficient heat for proper measurement, and the oxygen present in the tissue itself is sufficient for recovery, which is thus relatively rapid. The results of researches by this method will be considered in the next chapter.

The Lactic Acid Maximum.—The lactic acid maximum depends on the conditions of the experiment. Under most conditions formation of lactic acid ceases before all the glycogen store of the muscle has been removed. Excised frog muscles stimulated by single shocks to fatigue contain about 0·3 per cent. lactic acid; tetanic contractions yield rather less. The lactic acid maximum can conveniently be studied on minced muscle; the mincing initiates rapid break-down processes comparable with those which occur when the muscle is stimulated, and the formation of lactic

TABLE.

Treatment of Muscle.	Duration of Experiment.	Per gram of Muscle.				Lactic Acid oxidised mg.
		Gain or Loss of		Total O_2, mg.	Excess Oxygen, mg.	
		Lactic Acid, mg.	Glycogen, mg.			
Stimulated to fatigue .	30 m.	+3·15	—2·75	—	—	—
Recovery in oxygen . .	23 h.	—2·36	+0·92	1·48	0·84	1·39
	24 h.	—2·12	+0·97	1·22	0·68	1·14
	24 h.	—2·80	+1·44	1·45	0·73	1·36
Minced and kept in $\frac{1}{10}$ n. Na_2HPO_4 + $\frac{1}{1500}$ n. KCN.	25 m.	+0·88	−0·87	—	—	—
	2 h. 5 m.	+2·60	−2·95	—	—	—
	6 h.	+2·72	—2·80	—	—	—
Minced and kept in Na_2HPO_4 (*p*H 9) . . .	22 h.	+8·27	—	—	—	—
Minced and kept in 1 per cent. $NaHCO_3$ (*p*H 9·5) . .	22 h.	+2·4	—	—	—	—
Minced and kept in NaH_2PO_4 (*p*H 4·5)	23 h.	+0·97	—	—	—	—
Minced in phosphate (in H_2)	1 h. 40 m.	+3·83	−4·00	—	—	—
Minced in phosphate (in O_2)	1 h. 40 m.	+2·10	−3·05	0·43	—	0·40
Minced in phosphate + 0·3 per cent. arsenate (in H_2) .	1 h. 40 m.	+6·90	−6·50	—	—	—
Minced in phosphate + 0·3 per cent. arsenate (in O_2) .	1 h. 40 m.	+4·36	−6·00	0·57	—	0·53

acid continues until the maximum has been reached or, under certain conditions, until all the glycogen has been withdrawn from the tissue.

A higher lactic acid maximum is reached when the muscle is suspended in an alkaline solution, but the highest figures are reached when the solution is alkaline and also contains phosphates; under these circumstances, if oxygen is excluded, the whole of the glycogen is convertible into lactic acid. The Table on p. 145 gives some of the results from Meyerhof's numerous papers on the subject, and it will be seen that as much as 0·827 per cent. of lactic acid was obtained under these conditions, while immersion in an even more alkaline solution of bicarbonate gave only 0·24 per cent. of lactic acid. When placed in NaH_2PO_4, which gave an acid solution (*p*H 4·5), there was only 0·097 per cent. lactic acid produced.

The inferences from these experiments are that phosphates play an important part in the break-down process and that the process of formation tends to inhibit itself as soon as the lactic acid has accumulated in sufficient amount.

The importance of phosphates is further shown by the fact that if glucose, hexose-phosphate or glycogen is added to the muscle shortly before exhaustion of the store of carbohydrate, there is a further formation of lactic acid and corresponding destruction of the added carbohydrate. This does not happen in absence of added phosphates.

The rate of lactic acid formation during anaerobiosis in the stimulated or minced muscles is under these experimental conditions about four times as great as that calculated from the oxygen debt. When the oxygen usage of the minced muscle is increased by addition of arsenates or caffeine, there is an accelerated rate of disappearance of lactic acid, though complete restitution does not occur. In absence of oxygen, addition of arsenates or caffeine leads to a very rapid and extensive formation of lactic acid.

Certain substances can cause muscle to pass into a state of contraction, or even into rigor. Caffeine, chloroform, and

nicotine belong to these, and in all cases the strength and extent of the resulting contraction runs parallel with the amount of lactic acid generated. The gradual application of heat is another method (heat rigor) by which the muscle is caused to shorten, with production of lactic acid. It has been shown by Meyerhof and Himwich that when the glycogen content of the muscles of a rat has been reduced by an exclusive fat diet the lactic acid formed on rigor is much reduced, and the shortening and tension production of the muscles are also diminished. The fatigue maximum reached on electrical stimulation is also only about half that obtained from the muscles of carbohydrate-fed animals. These results suggest that carbohydrate is the source of muscular energy and of the lactic acid formation which is so closely associated with this. Further discussion of the matter will be found in Chapter VIII.

Relations between Respiration and Fermentation

It was shown by Pasteur in 1876 that yeast can thrive in the absence of oxygen, producing alcohol and carbon dioxide from sugar, whereas in presence of oxygen ordinary respiration is also carried on. Pasteur believed that fermentation replaced oxidation as a source of energy when oxygen was lacking; the idea that there is some similar relation between anaerobic and aerobic life in animal tissues is an old one, and recent work indicates that it is likely. In both instances carbohydrate is attacked, and there appear to be associated with the tissue enzymes some substances called co-enzymes. The co-enzymes can be removed from yeast, and also from muscle, by washing; washed yeast or muscle residue no longer ferments or respires, but on adding to either a boiled extract from either yeast or muscle there is a return of the power respectively to ferment or to respire.

Fermentation, with yeasts or bacteria, corresponds to lactic acid formation in muscle, and the effect of admitting oxygen to yeasts is to cause combustion of carbohydrate, at a rate which varies with different species of yeasts, accompanied by resynthesis to carbohydrate of intermediary products of fermentation. If this respiratory resynthesis is rapid enough (*e.g.* in *Torula*), then fermentation is suppressed by oxygen; if respiration is slow, then oxygen merely slows the fermentation, as in brewer's yeast. In some animal tissues, too, formation of lactic acid by glycolysis

may be so fast relative to the oxidative restitution that lactic acid continues to be formed even in oxygen, as shown by Warburg with malignant tumour tissues.

By extraction of muscle with KCl solution in the cold, Meyerhof has been able to prepare solutions of the glycolytic enzyme which rapidly convert various carbohydrates to lactic acid. Like yeast extracts, this can be separated by ultra-filtration into a heat-stable co-enzyme and a labile enzyme. The purified enzyme has been shown to be quite inert in absence of phosphate.

Influence of Phosphates.—The clearest parallel between respiration and fermentation is seen in the importance of phosphates for both processes. Harden and Young discovered that yeast juice can be separated by ultra-filtration (through a gelatine filter) into two portions, neither of which alone can ferment glucose. When the filtrate and residue were mixed, active fermentation of glucose could be effected. The residue contains the enzyme, while the filtrate contains two substances, or groups of substances, essential for fermentation ; one of these is the co-enzyme already mentioned ; the other is a phosphate. Addition of a soluble phosphate to a fermenting mixture of sugar with yeast juice caused an acceleration of the rate of fermentation, even up to twenty times its former speed.

Harden and Young found that the phosphate and the sugar had formed an ester, which they isolated and identified as a hexose-diphosphate, $C_6H_{10}O_4(PO_4R_2)_2$.

Robison also separated an unstable hexose-monophosphoric acid which is probably an intermediary product. The chemical events in alcoholic fermentation may probably be represented as follows :—

1*st Phase* (*rapid*)—

(*a*) $2C_6H_{12}O_6 + 2PO_4HR_2 = \underset{\text{(hexose-monophosphate)}}{2C_6H_{11}O_5(PO_4R_2)} + 2H_2O.$

(*b*) $2C_6H_{11}O_5(PO_4R_2) = 2CO_2 + 2C_2H_6O + \underset{\text{(diphosphate)}}{C_6H_{10}O_4(PO_4R_2)_2}.$

2*nd Phase* (*slow*).—In absence of co-ferment—

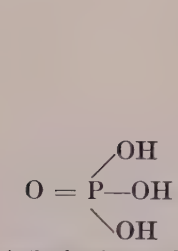
(*c*) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = 2CO_2 + 2C_2H_6O + 2PO_4HR_2.$

When the reaction begins to slacken, the hexose-diphosphate diminishes in amount, and the free phosphate increases again. This is either due to the reaction (*c*) shown above, or to a process of hydrolysis attributed to an enzyme called hexose-phosphatase.

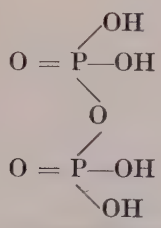
The Phosphorus Compounds of Muscle

Recent work has achieved noteworthy results in the study of the phosphorus compounds of muscle and has cleared away

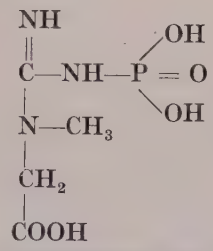
various misconceptions. As at present understood, the chief phosphorus compounds of muscle are orthophosphates, pyrophosphates, phosphagen, hexose-monophosphoric ester, hexose-diphosphoric ester and the nucleotides adenylic (and inosinic) acids. These are salts of the acids shown below :—



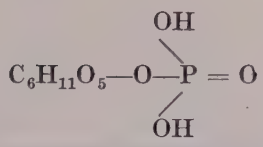
(orthophosphoric acid)



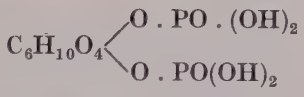
(pyrophosphoric acid)



(creatine-phosphoric acid)
possible formula

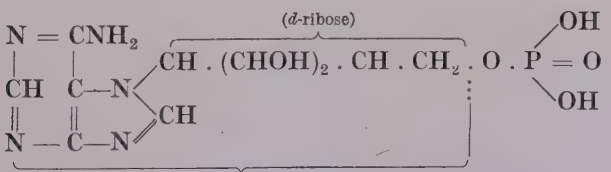


(hexose-monophosphoric acid)



(hexose-diphosphoric acid)

Adenylic acid is derived by condensation of adenine, *d*-ribose and phosphoric acid, and inosinic acid from deamination of adenylic acid, *i.e.*, from hypoxanthin, *d*-ribose and phosphoric acid.



(adenosine)

Adenylic Acid.

The following table, after Eggleton, shows the amounts of

various important substances present in resting muscle of the frog :—

	Per cent.
Glycogen	0·7
Lactic acid	0·02
" Free " creatine	0·095
" Combined " creatine	0·275
Ammonia	0·0015

Phosphorus Compounds (as P)—

In orthophosphoric acid	0·015	formerly called " inorganic "
,, creatine-phosphoric acid (phosphagen)	0·065	
,, hexose-monophosphoric ester	0·008	formerly called acid-soluble " organic "
,, hexose-diphosphoric ester	trace ?	
,, pyrophosphoric acid	0·025	
,, adenylic and inosinic acids	0·013	
,, other " acid-soluble " compounds	0·01–0·02	

Residual phosphorus insoluble in acid, is mainly lipide, and is only about 10 per cent. of the total phosphorus of the muscle.

Orthophosphate.—It will be noted that the orthophosphate of resting muscle is very small in amount—only of the order of 15 mg. per cent., and it may be even less in long-rested muscle. This explains why the phosphate, which was formerly believed to be the chief salt of muscle, does not nearly all diffuse out into the blood, the plasma of which contains inorganic phosphate in concentrations of the same order. Stella found, for frog's muscle, that when the outer fluid contained 8 mg. of phosphate-P per cent., equilibrium was obtained between the phosphate concentrations inside and outside, so that there was no passage of phosphate either into or out of the muscle.

Creatine-phosphoric Acid or Phosphagen.—It was observed

by P. and G. P. Eggleton that when inorganic phosphate is estimated in muscle extracts in alkaline solution about 20 mg. per cent. of phosphorus is found, but when the estimation is made in acid solution the content of phosphorus rises to about 90 mg. per cent. This led them to the discovery, also made independently by Fiske and Subbarow, of a new phosphorus compound which is unstable in acid solutions, and which subsequently proved to be a compound of phosphoric acid and creatine to which they gave the name of phosphagen. Creatine-phosphoric acid may be extracted from muscle, and when treated with acid, or with muscle enzymes and bicarbonate, it is split into phosphoric acid and creatine. It is most abundant in white muscle, less in red : cardiac muscle contains very little and plain muscle practically none. The discovery of phosphagen has given a reasonable explanation of the presence of creatine in muscle, but has necessitated the revision of many of our views on the chemical changes taking place in muscle.

It is certain, since very little creatine diffuses out of muscle, that most, if not all, of the creatine of resting muscle is not present in the free state, but is combined with phosphoric acid. In fact, Dulière has recently shown that total creatine and total directly estimable phosphate are present in muscle in equimolecular proportions, and that the " free " creatine in resting oxygenated muscle may be no more than 50 m.g. per cent. Creatine-phosphoric acid, however, is a readily diffusible substance, and might itself be expected to diffuse out from a muscle, but, as this does not happen, it is perhaps best to give the name phosphagen to the indiffusible form in which creatine-phosphoric acid appears to exist in the living muscle. Creatine-phosphate is hydrolysed to creatine and phosphate by enzymes present in chopped muscle. This conversion is accompanied by the evolution of about 11,000 calories per gram molecule of H_3PO_4 liberated.

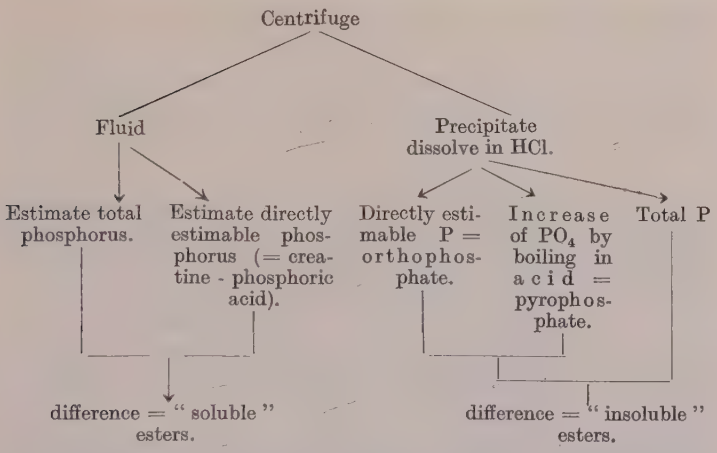
Pyrophosphoric acid was identified in muscle in 1928 by

Lohmann, who also obtained it from yeast. Pyrophosphoric is converted to orthophosphoric acid by boiling with dilute acids, but is fairly stable in neutral or alkaline solutions. Muscle and other tissues, however, contain an enzyme which, according to Kay, is most active at *p*H 7·2–7·8, and which rapidly carries out the conversion. Pyrophosphate is not free as such in the muscle, since it does not diffuse out of it, but, according to Lohmann, is held combined with adenylic acid. One molecule of adenylic acid compound can yield one molecule of pyrophosphoric acid (two atoms of phosphorus) and one of orthophosphoric acid.

Hexose-phosphoric Esters. — Of these, normal resting muscle appears to contain only the monophosphoric ester. This can be acted upon by enzymes extractable from muscle, and converted ultimately into lactic and phosphoric acids; in the conversion of carbohydrate to lactic acid, phosphoric ester stages are passed through in muscle as in the fermentation process in yeast: the hexose-monophosphate first formed breaking up quickly into a diphosphate and lactic acid: $2C_6H_{11}O_5(PO_4R_2) = 2C_3H_6O_3 + C_6H_{10}O_4(PO_4R_2)_2$, and then the diphosphate slowly breaks down into phosphoric and lactic acids (*i.e.*, we get $C_3H_6O_3$ instead of, as in yeast, $CO_2 + C_2H_6O$).

Phosphoric Esterase.—There is present in many tissues an enzyme, phosphatase, or phosphoric esterase, which can effect the hydrolysis of various phosphoric esters, *e.g.*, glycerophosphate, hexose-diphosphate, nucleotide (which is converted to nucleoside + phosphoric acid). It is probably identical with pyrophosphatase, though it acts best on phosphoric esters at *p*H 8·8–9·3.

Estimation of the Phosphorus Compounds of Muscle. — The estimation of the various phosphorus fractions in muscle can be carried out by a method devised by the Eggletons, of which the following is a brief summary: (1) extraction of the muscle with N/4 trichloracetic acid; (2) extract neutralised with $Ba(OH)_2$ to *p*H ·9; (3) then as follows:—


Centrifuge
Fluid
Precipitate dissolve in HCl.
Estimate total phosphorus.
Estimate directly estimable phosphorus (= creatine - phosphoric acid).
Directly estimable P = orthophosphate.
Increase of PO_4 by boiling in acid = pyrophosphate.
Total P
difference = " soluble " esters.
difference = " insoluble " esters.

The " soluble " esters probably consist of hexose-monophosphoric acid. The " insoluble " esters probably represent the *o*-phosphoric acid originally combined as adenylic pyrophosphate compound, the pyrophosphate having been previously split off from it by the acid. It has therefore half the value of the pyrophosphate phosphorus, since pyrophosphoric acid contains two atoms of phosphorus.

Changes in Phosphorus Distribution of Muscle during Activity.—When muscles are stimulated to the point of fatigue, it is found that the phosphate fractions in the fatigued muscles compare with those of control batches of fresh resting muscle somewhat as shown in the table on p. 154 :—

It will be seen from this table that there is a very distinct increase in the percentage of orthophosphate and a corresponding, or even slightly greater, diminution in the creatine-phosphate or phosphagen fraction. Of the other fractions the only one that shows much change is the " soluble " ester fraction which shows a $7\frac{1}{2}$ per cent. increase. The pyrophosphate and insoluble esters show no appreciable change. This may be interpreted as meaning that there is a conversion of phosphagen into creatine and phosphate, and

further, that there is an increase in the soluble esters (apparently at the expense of the orthophosphate). The adenylic pyrophosphate compound is apparently unaffected, so that there is no change in pyrophosphate (insoluble esters).

When muscle is kept for a few hours in anaerobic condi-

The effect of rapidly induced fatigue on skeletal muscles of frog. (G. P. and P. Eggleton.)

The total phosphate values are given in milligrammes of phosphorus per 100 g. of muscle; the five fractions are expressed as percentages of the total phosphorus.

	Total phosphate.	Orthophosphate.	Phosphagen.	Pyrophosphate.	"Soluble" esters.	"Insoluble" esters.
1. Resting	142½	24	40	20	5	11
Fatigued	142½	38	18	23½	12	8½
Change	—	+14	−22	+ 3½	+ 7	− 2½
2. Resting	105	20	39	21½	5½	14
Fatigued	105½	37½	16½	24	10	12
Change	—	+17½	−22½	+ 2½	+ 4½	− 2
3. Resting	121½	19½	40	24	4½	12
Fatigued	120½	40½	5	24	15	15½
Change	—	+21	−35	0	+10½	+ 3½
4. Resting	134	18½	49	20½	2	10
Fatigued	125½	53½	4½	18½	11	12½
Change	—	+35	−44½	− 2	+ 9	+ 2½
5. Resting	138½	20½	47½	21	1	10
Fatigued	138½	34½	26	21	7½	11
Change	—	+14	−21½	0	+ 6½	+ 1
6. Resting	112½	15	42½	22	9	11½
Fatigued	111½	38	12	21½	15½	12
Change	—	+23	−30½	− ½	+ 6½	+ ½
Mean change	—	+21	−29½	+ ½	+ 7½	+ ½

tions, phosphagen disappears from it, and orthophosphate takes its place.

There is at first little change in the pyrophosphate and insoluble ester fraction, but after the phosphagen has been completely exhausted these two fractions, pyrophosphate and insoluble esters, decline rapidly, and ultimately dis-

appear. The soluble esters show a steady increase throughout the period of anaerobiosis. These results are exemplified in the following table :—

The effect of prolonged oxygen lack on the distribution of phosphorus in the skeletal muscles of the frog. (G. P. and P. Eggleton.)

The gastrocnemii (G.), graciles (Gr.) and semi-membranosi (S.) from the left (L.) and right (R.) legs of two frogs (1 and 2) were kept in moist hydrogen at room temperature for the stated time. The total acid-soluble phosphorus is given in mg. per 100 g. of muscle ; the five fractions are expressed as a percentage of this figure.

Time in hours.	Muscle.	Total.	Ortho-phosphate.	Phos-phagen.	Pyro-phosphate.	"Soluble" esters.	"Insoluble" esters.
0	1 Gr. L.	128·8	13·1	53·3	21·6	2·6	9·6
0	1 S. R.	128·0	15·1	49·4	28·5	4·3	2·7
4	1 G. L.	129·2	31·8	30·6	21·4	5·7	10·5
4	2 Gr. R.	135·1	28·2	36·3	24·0	5·5	5·8
19	2 S. L.	141·1	47·8	12·2	22·4	8·4	9·4
19	2 G. R.	148·4	46·2	14·2	22·5	6·6	10·6
26	1 Gr.R.	141·9	51·5	9·2	19·0	9·1	11·3
26	1 G. R.	129·5	62·6	6·2	14·4	7·3	9·4
43¼	2 G. L.	133·0	65·1	3·0	13·4	7·5	10·9
43¼	1 S. L.	136·2	83·7	0	2·9	8·9	4·4
50½	2 S. R.	144·0	90·6	0	2·8	9·4	0
50½	2 Gr. L.	174·0	89·7	0	0	10·3	0

On recovery in oxygen for one hour from moderate fatigue, the inorganic phosphate is found to be restored again to phosphagen.

The amount of creatine which is set free in the muscle during contraction, or during a period of oxygen lack, is apparently equivalent to the amount of orthophosphate which appears and of phosphagen which vanishes. During the recovery process creatine disappears again, with the orthophosphate, as phosphagen increases. There is, however, no fixed relationship between the amounts of orthophosphate and of lactic acid which are produced, though usually for

short tetani of fresh muscle there will be about two molecules of lactate to one molecule of orthophosphate. It has been observed by Nachmansohn that the phosphate production (phosphagen break-down) is much less in a curarised than in an uncurarised muscle, there being in the curarised muscle somewhat less than 1 molecule of orthophosphate appearing for each molecule of lactic acid formed. It has been claimed that immediately after a tetanus, even under anaerobic conditions, there is a rapid reformation of phosphagen.

A curious phenomenon is seen when the muscle is incubated with fluoride solution; under these conditions there is a disappearance of inorganic phosphate, and also, when they are added to the muscle, of glycogen and additional phosphate. It is interpreted as due to the conversion of the carbohydrate and phosphate into the hexose-diphosphoric ester. This is apparently because the diphosphate is normally produced in the course of enzymic processes which take place in the muscle, but its further glycolysis is inhibited by the presence of the fluoride. The formation of the diphosphate therefore continues, until ultimately all the phosphate, or glycogen, or both of them, becomes exhausted.

The manner in which fluoride acts in preventing glycolysis, *i.e.* conversion of carbohydrate into lactic acid, is obscure. Dickens and Šimer have produced evidence that it is by forming an inactive compound with some substance essential to glycolysis. In some tissues, at all events, they find that fluoride inhibits aerobic, as well as anaerobic, glycolysis, while in others (rat testis) it does not inhibit anaerobic glycolysis.

It may be convenient provisionally to attempt to harmonise these facts with those of fermentation as viewed by Meyerhof, and to regard phosphagen as a reservoir for phosphate readily available for esterification. First glycogen would pass (*viâ* hexose ?) to the hexose-monophosphate; which would then break up into lactic acid and the stable hexose-diphosphate as already explained.

The latter is then broken up by hydrolysis again, to give

phosphate and hexose or lactic acid, but in presence of fluoride this is inhibited so that there is ultimately a large formation of hexose-diphosphate.

Under aerobic conditions the phosphagen split up on contraction is quickly re-formed, and it is conceivable that its resynthesis bears some relation to the initiation of the oxidative removal of lactic acid, *e.g.*

$$\begin{aligned} &8\,C_3H_6O_3 + 8\,HR_2PO_4 + C_6H_{12}O_6 + 6O_2 \\ &= 4C_6H_{10}O_4\,(PO_4R_2)_2 + 6CO_2 + 14H_2O \\ &= 4(C_6H_{10}O_5) + 8HR_2PO_4 + 6CO_2 + 10H_2O \end{aligned}$$

in which equations we get formation of four equivalents of glycogen, with combustion of one of hexose.

In view of the fact that neither phosphagen, creatine, nor phosphate are present in more than traces in plain muscle, however, it would seem safest to regard their functions in contraction as accessory rather than fundamental.

Ammonia Production.—Though a small increase of nitrogenous metabolism has been claimed to accompany or follow the carrying out of muscular work in the intact animal, little attention has been paid to the nitrogenous changes in isolated muscles, partly because of lack of appropriate methods, partly, no doubt, because the carbohydrate and phosphate cycles have so largely occupied the stage and for a time appeared to be competent to explain the chemical and myothermal phenomena.

Recently, however, Parnas, Embden and others have studied the ammonia content of muscle and found that there is a small but unmistakable increase in ammonia as a result of injury or fatigue. It is claimed that this ammonia arises from adenylic acid, one of the five nitrogen atoms of which is removed as ammonia, and it is stated in support of this that when adenylic acid is added to muscle pulp, about a fifth of its nitrogen is so liberated, owing to the action of a deaminase, the adenylic acid being thereby converted to inosinic acid.

During recovery the adenylic acid is believed to be re-

formed, though there is a difference of opinion as to the manner in which this occurs: some think the ammonia vanishes again, others that it does not, but that the reamination occurs from another source.

Recently Lehnartz has stated that in presence of added adenylic acid, orthophosphate disappears, and is converted into pyrophosphate (? combined with adenylic acid) and phosphagen.

If these and various other claims are substantiated by further work, the focus of biochemical interest in muscle may readily be moved from the carbohydrate cycle to the part played by adenylic acid, and the possibility of interesting developments in this direction is not lessened by the recent demonstration by Drury and Szent Gyorgi that inosinic acid is a substance having certain powerful physiological actions: injection of a minute amount into the circulation causing a transient complete heartblock.

The Inexcitable Muscle.—It was noticed by Hermann in 1867 that sartorii suspended in oxygen rapidly lose their excitability, though gastrocnemii remain irritable longer in oxygen than in air. This curious phenomenon of loss of excitability, which is often seen in class work, has recently received an explanation from the work of Dulière and Horton. They found that if a frog's sartorius muscle was dissected straight off the body, without any application of Ringer's solution, there was, in the course of an hour or two, a loss of excitability. This loss of excitability was also seen when the muscle was suspended in nitrogen, or in air, or air containing 5 per cent. carbon dioxide, or immersed in liquid paraffin. The excitability can be restored again by immersing the muscle in Ringer's solution, and in a muscle so restored, or in one dissected out originally with immersion of the preparation in Ringer's solution, there is for a long time no loss of excitability when the muscle is subsequently kept in moist gas. Various other solutions are capable of restoring excitability, *e.g.*, isotonic NaCl.

A similar loss of excitability can be demonstrated in muscle soaked in Ringer's solution containing about four times the usual concentration of potassium, and in such cases recovery can be effected by washing the muscle in ordinary Ringer's fluid, or in Ringer's fluid containing up to three times the normal potassium concentration.

It is suggested that the onset of the inexcitable state in a muscle freshly dissected out and suspended in moist gas may be due to small quantities of potassium diffusing out from the interior of the fibres to the interspaces between them : it was actually demonstrated that potassium does leave an isolated muscle in sufficient amount to render the muscle inexcitable. This is washed away when the muscle is perfused with, or suspended in, Ringer's solution. In support of this hypothesis, it is shown that washing the inexcitable muscle in Ringer's solution containing more than three times the usual potassium concentration fails to restore the excitability of the tissue, though washing in ordinary Ringer's solution, or plain isotonic NaCl solution, will do so. Horton finds that the rate at which potassium leaves the muscle and the rate at which the inexcitable state supervenes are alike accelerated by damage, and by the presence of acids, such as carbon dioxide, which are capable of penetrating the fibre. It is assumed that in a large mass of muscle the amount of lymph present is relatively great, so that unless the muscle be treated with carbon dioxide or grossly damaged, the onset of the inexcitable state may be so slow as usually to escape notice. These experiments indicate the importance of the existence of a state of balance between the concentration of the different ions inside and outside the muscle fibre.

Theories of Muscular Contraction

It seems premature at the present time to speculate as to the manner in which muscular contraction is brought about

and as to the part which is played in the causation of the contraction by the chemical events which have just been discussed.

That there is a close parallel between the chemical happenings and the onset of the contraction is, however, quite evident. The majority of investigators are of the opinion that the liberation of lactic acid evokes the contraction. This opinion is supported by the observation that the isometric coefficient for lactic acid

$$\left(= \frac{\text{Kg. tension} \times \text{cms. muscle length}}{\text{mg. Lactic acid}}\right)$$

has a fairly constant value of about 100, or, in grammes and centimetres,

$$\frac{(\text{grammes tension}) \times (\text{cms. length})}{\text{grammes Lactic acid}} = 10^8,$$

(*i.e.*, 1 gramme-cm. of tension length $= 10^{-8}$ gramme lactic acid), and by facts of the origin and subsequent removal of lactic acid. It is certain that, if lactic acid in sufficient amount to render the solution acid is added to Ringer's solution in which a frog's gastrocnemius is immersed, the muscle shortens and passes into a condition which resembles that of rigor. Since in the production of rigor there is a large production of lactic acid, it has been usual to assume that the shortening of rigor is also due to the formation of lactic acid. It was shown long ago by Claude Bernard, however, that the onset of rigor was correlated, not necessarily with the attainment of an acid reaction, but with the complete disappearance of glycogen from the muscle, and recent investigations of Hoet and Marks have tended to revive this view by showing, as Claude Bernard did, that when the glycogen has all gone rigor sets in, even though the muscle may still be alkaline. When the glycogen of the muscles is completely removed by large doses of insulin (*vide* p. 428) the animal's muscles pass at once into rigor as it dies.

It would be anticipated that a theory of muscular con-

traction which served to explain the phenomena in striated muscle would be transferable to the somewhat similar phenomena of plain muscle, but at the present time this does not seem to come up to expectations. I have made determinations of the lactic acid content of plain muscle from various sources under a variety of conditions, and found, on the whole, that lactic acid is produced under similar conditions to those which lead to its formation in striated muscle. Yet, as Underhill and I have shown, the effect of addition of reasonable amounts of lactic acid to plain muscle is most commonly to cause not contraction, but relaxation. It is true that we cannot infer much of what occurs inside the fibre by steeping it in a solution of any sort, nor does it seem justifiable to draw conclusions from such an experiment for one tissue and reject them for another. Various explanations of the discrepancy might be given, but as none of them is to my mind at all satisfactory, it seems better merely to state the fact and await the explanation. A further singular fact is that caffeine, which accelerates lactic acid formation in striated muscle, actually delays it in plain muscle ; arsenates and chloroform, however, hasten lactic acid formation in both varieties.

If a production of lactic acid leads to contraction, how are we to explain the fact that, when the lactic acid is not removed, the muscle can still relax again ? The explanation which is offered is that the lactic acid is generated at certain sites in the muscle fibre (let us suppose in the anisotropous material), and at these sites produces the physical alterations which eventuate in contraction. This is a momentary affair, and very soon the lactic acid diffuses away again into the general substance of the fibre and is neutralised by the buffers, which are very abundant there. Chief among these buffers are salts of the proteins with bases ; when the muscle is fatigued the proportion of alcohol-soluble inorganic material (bases present as lactates) have been shown to be much increased. It is only after numerous contractions

in absence of oxygen that sufficient lactic acid accumulates to affect the hydrogen ion concentration permanently, and so to produce the state of contracture.

As to the manner in which the lactic acid, when it has been liberated, brings about the state of contraction, there may be said to be four theories, which may be called the surface tension theory, the osmotic theory, the swelling theory and the theory of liquid crystals. According to the first of these, the lactic acid raises the surface tension at the surfaces in which it is produced; if these surfaces are not spherical already, they tend to become so, and thus to induce shortening. Meyerhof has shown that there is a constant ratio between the isometric tension developed by unit length of muscle and the weight of lactic acid formed, and Hill, by recalculating these results in C.G.S. units, finds that when a force of 1 dyne per cm. length of muscle is set up there is a liberation of $13{\cdot}1 \times 10^{-12}$ grams of lactic acid. This value agrees well with that calculated by Hill from his own thermal data (viz., 16×10^{-12}). Further calculations show that even if this amount of lactic acid were spread out in a layer only one molecule thick, the alterations of surface tension necessary to develop a force of 1 dyne would have to be much greater than any that it could produce, and hence the surface tension theory is provisionally regarded as untenable.

The osmotic theory presupposes a semi-permeable membrane either at the surface of the fibre or somewhere within it. Production of lactic acid or other substances of small molecular weight within this membrane would lead to attraction of water from the surroundings, and so to local swelling of the enclosed structure, which could conceivably lead to shortening. In support of this view it is alleged that, when viewed under the microscope, the clear stripe of the fibre is seen to diminish, while the dark stripe increases in volume; further, the fatigued muscle, when immersed in hypotonic solutions, does actually gain in weight faster than the fresh tissue. But since there has

been an undoubted production in the active tissue of substances of low, from precursors of high, molecular weight, this is what might have been expected to happen.

The third theory, that of swelling, is more difficult to appraise. This theory is at present favoured by Embden and his colleagues, whose opinion it is that the production of lactic (and phosphoric) acid leads to an uptake of water by the cell colloids, while removal of these catabolites leads to shrinkage of the cell substance by a reversal of the process. They also believe, which seems by no means unlikely, that excitation of the muscle is accompanied by profound alterations in the permeability of the surface membranes.

The theory of liquid crystal structure has recently been advanced by Garner, in the attempt to find a theory of surface action which did not necessitate the occupation by the lactic acid of the whole of the surface concerned. If it be assumed that there is a film of liquid crystals in or upon the anisotropic substance, then the film might be caused to expand or contract by alteration in the angles of the lattice, which change, if it occurs, might be attributable to the effects of lactic acid. A somewhat similar theory is advanced by Clark ; she assumes that a liquid crystalline body is present in the anisotropous substance, and that this passes abruptly into the solid crystalline state as a result of increase of hydrogen ion concentration ; the calculated cohesive force between the crystal layers is of the same order as the force exerted by a muscle fibre on contraction.

It cannot be said that the exploration of the relationship between lactic acid and contraction has up to the present led to any satisfactory solution of the problem. It may be that, after all, the lactic acid production is only a collateral phenomenon, and that a more fundamental factor is to be sought along other lines, one of which is suggested by the relation between rigor mortis and glycogen depletion, referred to on p. 160. It is not unthinkable that the physiological state of the muscle fibre depends on the equilibrium con-

ditions of a tissue complex of which glycogen is a component, and that the appearance of lactic acid is merely one of the outward signs of an alteration in the equilibrium conditions of this complex.

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CHAPTER VII

PHYSICAL ASPECTS OF THE PHYSIOLOGY OF MUSCULAR CONTRACTION

1. The Mechanical Aspect of Contraction

When a living muscle is appropriately stimulated, it passes, after a brief latent period, from the resting to the contracted state. By contraction is understood the tendency of the muscle to shorten, owing to a change in its elastic properties, and under certain conditions the muscle does actually shorten, thereby performing work. Not infrequently, however, the muscle is unable to shorten, and the effect of the contraction is shown only by the exertion of a tension upon its fixed extremities. This is the isometric contraction.

Muscle is a machine by which chemical potential energy is ultimately converted into the potential energy represented by the tension set up in the muscle fibres in the act of contraction ; in consequence of this the muscle acts as though it were a stretched elastic structure, capable, therefore, of exerting a pull on its extremities. If the muscle is allowed to shorten, this potential energy is partially converted into work. Moreover, when the muscle shortens and does work, an extra amount of energy is mobilised which would not have appeared if the contraction had been purely isometric. A muscle which is allowed to shorten on contraction has thus a larger source of elastic or other potential energy to draw upon than one which is not allowed to shorten. This new conception of the course of a contraction is well expressed by Fenn, who suggests that the contraction is analogous rather to the winding up of an anchor chain by a windlass

than to the lifting of a weight by the energy of a stretched spring.

It is better, as A. V. Hill has suggested, to regard the potential energy set free, rather than the actual work done, as the mechanical end product of the muscular contraction.

The theoretical maximum work (W_o) is that which would be done if all the potential energy set free on contraction could be converted without loss into work; it is always much greater than the *realisable work* (W). The magnitude

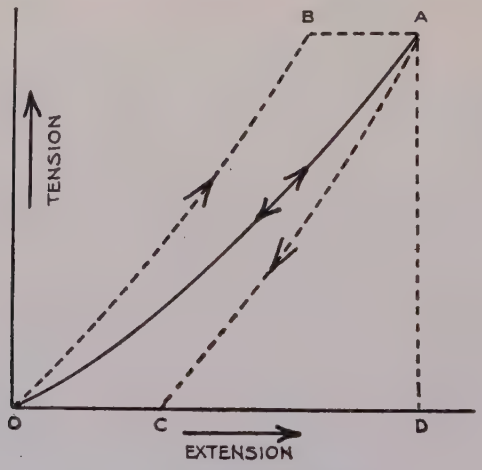


Fig. 46.—Stress-strain diagram.

of the latter is determined among other things by the manner and speed of the shortening, as will presently be described, while the former depends only on the stimulus and on such factors as the temperature, length, amount of shortening, and condition of the muscle fibres.

The Theoretical Maximum Work.—If we could regard the potential energy of the muscle in the contracting condition as comparable with that of a stretched spring, the theoretical maximum work of the muscle could be derived from the "stress-strain diagram." In this graph the force exerted by

the stretched (contracted or resting) muscle is plotted against the distance through which it has been stretched, and from the area of the curve the potential energy of the stretched muscle is found. In practice, however, the tension developed during stretching is greater than that at the same length during release. This is because muscle shows the property of " after-extension " so strongly that stress-strain diagrams could only give the theoretical maximal work if the extension were carried out with infinite slowness.

Thus, suppose that in Fig. 46, if the muscle is extended with infinite slowness by the gradual application of a load, the curve OA is given, and when the load is removed, the curve AO. But if the load is more suddenly applied a curve more like OBA is produced, in which the muscle first extends to B, and then slowly, without the application of further load, to A. Similarly, when the load is suddenly removed the muscle shortens from A to C, and then more slowly to O, its initial unloaded length. The potential energy of the stretched muscle corresponds to the area OAD ; that of the muscle at the moment of rapid extension to B is equal to the area OBAD, and the work obtained from it on rapid unloading to the area ACD ; the difference, viz. the area OBAC, corresponds to the work which has been lost as heat, when the muscle is suddenly stretched and suddenly released again.

The theoretical maximum work of a muscle in isometric contraction can be determined by the method of a stress-strain diagram used by Fick, and recently by Hill and by Meyerhof, in the following way: The muscle is first made to contract isometrically by attaching it by a thread to an isometric lever. At the next contraction the attachment of the muscle is moved nearer to the isometric lever, so that the thread is slack ; the muscle then contracts isotonically until the thread is drawn tight, after which its contraction becomes isometric. This is repeated, with greater and greater approximation of the fixed end of the

muscle to the lever, until finally the whole contraction is isotonic. The tensions of the resting muscle at different degrees of extension are then taken, and the results plotted in the form of a graph, as in Fig. 47. In this figure OR represents the natural length of the resting muscle, and OC the natural length of the contracted muscle. The line RR′ is the relation between tension and length of the resting muscle, and CC′ that between tension and length of the contracted muscle, as recorded on the isometric lever. The

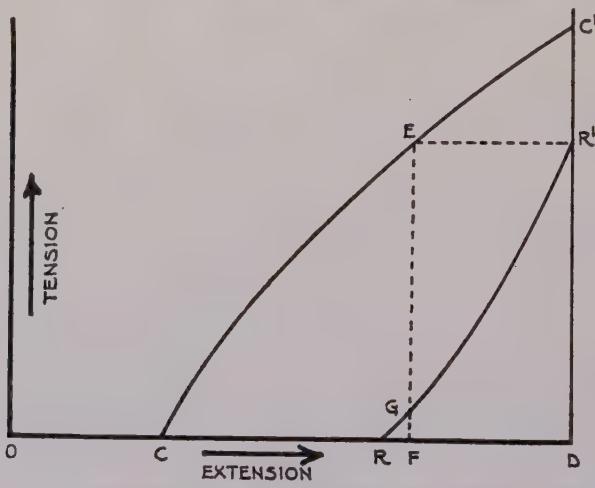


Fig. 47.—Stress-strain diagram of relaxed and contracted muscle.

area CC′D is the potential energy of the contracted muscle, and RR′D that of the resting muscle. Hence the amount of energy which the muscle has at its disposal as a result of the isometric contraction is the difference between the two, viz., the area CC′R′R. If the muscle had performed a purely isotonic contraction, under an initial tension of DR′, it would contract only to the length E, instead of to C, and the work then done, viz., C′EFD, would be largely due to the portion R′GFD, which is due to the potential energy of the resting stretched muscle, and only partly to the excitation, which is

only responsible for the small remainder C′EGR′. (This argument, however, only applies if the isotonic contraction has no more energy at its disposal than the isometric, of which there is now grave doubt.)

Tension and Length of Fibre.—A. V. Hill has found that there is a definite relation between the elastic potential energy set free in isometric contraction, *i.e.*, the area C′CRR′ of Fig. 47, and the length of the muscle, and the maximum tension set up on isometric contraction. This means that, if we regard the isometrically contracted muscle as a stretched spring, a definite fraction of the energy utilised in contraction appears as mechanical potential energy of the contracted muscle. The relation is different for different muscles, but for single twitches of the frog's sartorius was $W_o = Tl/6$, where T = the isometric tension, and l = the length of the muscle. Thus if the muscle had a length of 3·3 cm., and the tension set up was 44·8 grams, the value would be $\frac{3{\cdot}3 \times 44{\cdot}8}{6}$ = 24·6 gram-cm. Knowing the mechanical equivalent of heat to be that 1 gram-cm. = 23·4 microcalories, we can at once express the potential energy in terms of heat units ; in the above instance it would be equal to 24·6 × 23·4 = 576 microcalories. Hill found that the actual heat produced by a muscle in isometric contraction was the same as that calculated in the above manner from the potential energy, and therefore he inferred that in the act of isometric contraction (not including the recovery process) chemical potential energy (C) was completely converted into elastic potential energy, which then passed quantitatively into heat during relaxation ; *i.e.*, $C = W_o = H$.

Similar results are obtained by Hill's calculation from data by Meyerhof. We have seen that the isometric coefficient of lactic acid for a series of twitches is about 1×10^8, *i.e.*,

$$\frac{\text{(grams tension)} \times \text{(cms. muscle length)}}{\text{grams lactic acid produced.}} = 1 \times 10^8,$$

or, 1 gram-cm. (tension-length) = 10^{-8} gram lactic acid. (I.)

Meyerhof has also shown that in the anaerobic liberation of 1 gram lactic acid, 385 calories of heat are liberated, whence

$$385 \times 42 \times 10^3 \text{ gram-cms.} = 1 \text{ gram lactic acid,}$$

or, 1 gram-cm. (heat) $= 6{\cdot}14 \times 10^{-8}$ gram lactic acid. (II.) From (I.) and (II.) we can deduce that

$$\frac{Tl}{H} = 6{\cdot}14$$

Direct observation of $\frac{Tl}{H}$ by Hill gave average results of 6·16 for anaerobic contractions.

The Realisable Work.—Let us assume for the moment, as until quite recently it has been assumed, that the total potential energy set free on contraction is the same whether the muscle contract isotonically or isometrically, *i.e.*, whether it be allowed to shorten or not.

The tension energy (W_o) which is produced in a muscle on contraction can be made to yield work under appropriate conditions, but the conversion into work is always more or less incomplete, for the following reasons : (*a*) if the load is too great, the shortening of the muscle is not completed ; (*b*) if the load is too small, the full force of the muscle is not utilised ; (*c*) relaxation often sets in before the shortening is completed. The energy which is not utilised in doing work is converted into heat. In isometric contraction it is all so converted.

If the muscle be allowed to work against the inertial resistance of a mass instead of pulling a fixed load, and if the mass be varied so that shortening is just completed before relaxation begins, the loss due to these factors is eliminated, and the realisable work approaches the theoretical maximum work, though, for reasons to be given later, it can never actually equal it.

The opposition of the force of the contraction by the inertia

of a mass depends on the principle that at every stage of the shortening the force exerted by the muscle is opposed by a force which it can only just overcome. At the commencement of contraction the muscle has to overcome the inertia of the mass ; and its energy is used in overcoming this reaction, instead of in causing a rapid shortening, in which much of it would be lost ; later, when its force is less, it has merely to accelerate its movement. Several instruments have been devised for achieving this end, and we will

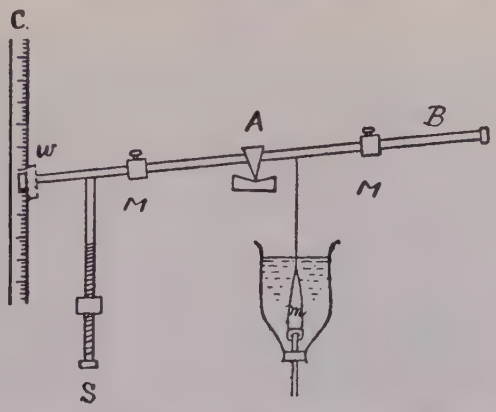


Fig. 48.—Inertia lever for frog's muscle. (Doi, *Journal of Physiology.*)

consider two of those used by A. V. Hill, the one for frog's, the other for human, muscles.

The arrangement for frog's muscle is shown in Fig. 48. A long steel rod, B, is balanced on a knife edge, A, and carries two masses, M (say, 50 to 100 grams each), placed at such distances from the knife edge that the lever is balanced. The muscle pulls on one arm of the lever at a distance, a, from the axis. The moment of inertia of the system about the axis is equal to MK^2, where M is the total balanced mass and K its radius of gyration. The movement is similar to that of a balanced mass, MK^2/a^2, pulled directly by the muscle, a being the distance of the

application of the muscle from the axis. The " equivalent mass " MK^2/a^2 can be varied by varying the distance of the masses or the distance of the attachment of the muscle from the axis. At the end of the rod is placed a relatively small weight, w, of a suitable mass (say, for frog's sartorii between 0·5 and 5 grams), which keeps the relaxed muscle extended to a given length determined by the adjustment of the screw S, which supports the rod. When the muscle contracts, it lifts the weight w to a height, h, which is measured on the scale C, and the work done is equal to wh. The values of the effective mass and of the weight w are altered until the maximum value of the work done (wh) is found, as illustrated in the following table :—

Maximal Work of Frog's Sartorii.

(From Doi, *Journal of Physiology*, 1920, **54**, 336.)

Value of w in grams.	Work done (wh) by muscle (grm.-cm.) when Effective mass (grams) = 479	881	1314
0·9	6·93	7·56	7·38
1·1	7·37	7·70	7·48
1·3	7·15	7·41	7·20

The maximum work done (7·7 gram-cm.) is that when $w = 1{\cdot}1$ grams, and the effective mass = 881 grams, but a deduction has to be made from this to allow for the potential energy of the extended resting muscle, and this correction reduces the maximum realisable work to 6·64 gram-cm.

Doi found in these experiments that the maximum realisable work (W) bore a constant relation to Tl (T being the tension set up on *isometric* contraction, and l the length of the muscle), viz. : $W = Tl/20$. The muscle is thus only able to convert a fraction (one-third to one-fourth) of its elastic potential energy (theoretical maximum work = $Tl/6$) into realisable work.

For human muscles the same principle is adopted of

opposing to the muscle at each stage of its contraction a force which it can only just overcome, but the type of apparatus used for the frog muscle could not easily be employed, because the balanced mass required would have to be of the order of half a ton. Instead, a heavy flywheel (35 kg.) on ball bearings is used, carrying a pulley cone. A string is wound round one of the pulleys, so that a gearing effect is obtained, and the subject, standing as in Fig. 49, pulls the string, using only the biceps and brachialis anticus muscles.[1]

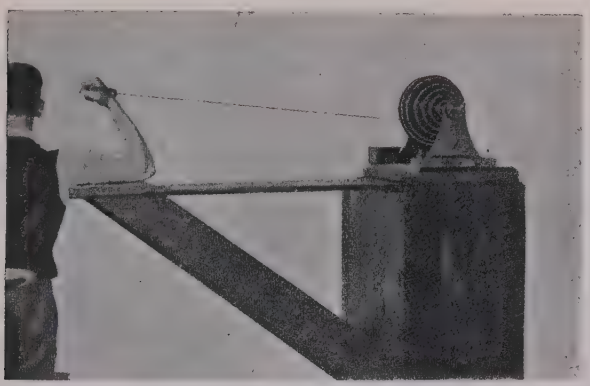


FIG. 49.—Inertia flywheel for human muscle. (Hill, *Journal of Physiology*.)

When the string unwinds, it drops off the cone, leaving the wheel rotating. The speed of rotation thus imparted to the wheel is measured. The wheel must be calibrated to find the value of the work which must be done to produce rotation at given speeds when the string is pulled. A calculation

[1] Hansen and Lindhard (*Journ. Phys.*, 1923, **57**, 287) claim that the muscles employed also include the brachio-radialis, extensor carpi radialis longus and pronator teres. They further contest Hill's statement that W approximates to W_o under any circumstances, because they maintain that in addition to loss by viscous friction, there is in prolonged contractions a loss of energy due to fatigue. They propose to calculate W_o from measurements of the maximum pull on the string, and by this method find it much higher than from Hill's method.

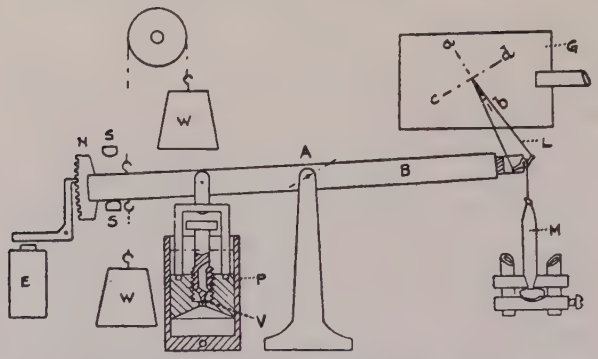


FIG. 50.—Diagram of the Levin-Wyman Ergometer.

gives the "equivalent mass." By using different pulleys on the cone the gearing effect, the moment of inertia, and therefore the equivalent mass, can be varied. In the apparatus used by Hill there were eight pulleys, allowing of a variation of the effective mass of the inertial resistance from 11·3 to 579 kg.

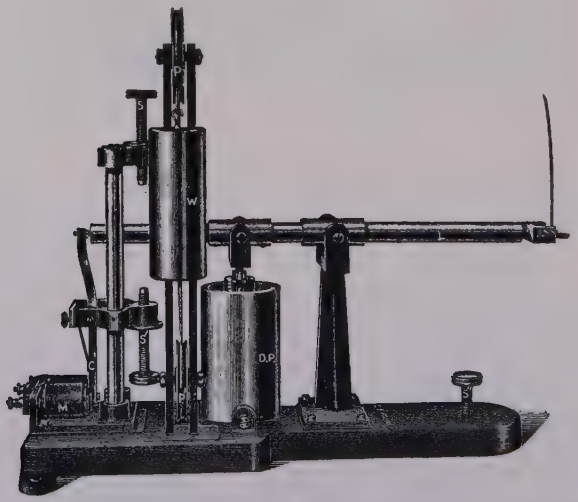
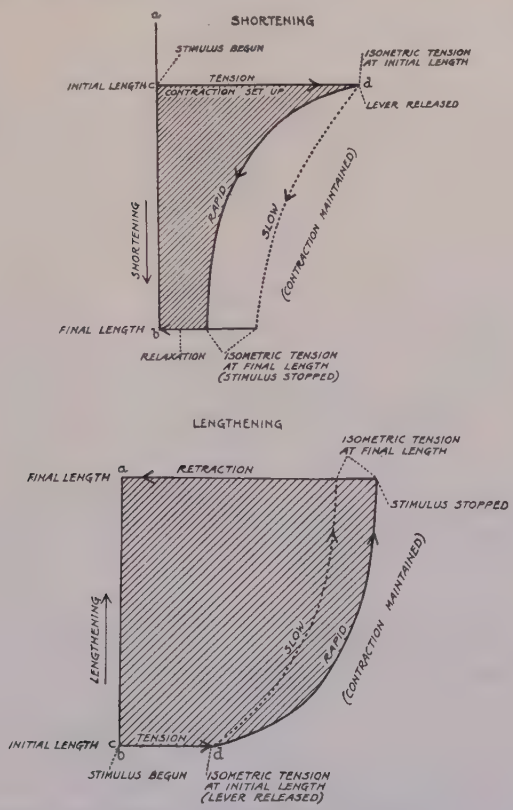


FIG. 51.—The Levin-Wyman Ergometer. (C. F. Palmer & Co.)

As with isolated frog's muscles, it was found that the maximum realisable work could approximate to, but never reach, the theoretical maximum work.



FIGS. 52 & 53.—To illustrate the principle of tension-length diagrams in tetanic contractions for shortening and lengthening.

A valuable instrument for studying the work done by a contracting muscle is the ergometer introduced by Levin and Wyman. This apparatus enabled them to study the work done by (or on) a muscle when it shortened (or was stretched) between known constant limits and at a known

speed. The method consists essentially in recording along approximately rectangular co-ordinates the simultaneous changes in tension and length as the muscle shortens or is stretched, and thus writing a tension-length diagram, the area of which gives the work done under the prescribed conditions.

The apparatus is constructed as follows : The muscle, fixed at one extremity, is attached to one end of a balanced beam B (Fig. 50), which can be fixed or released at will by an electromagnet E. When the beam is rocked, as it would be if the muscle M were supposed to shorten without increasing its tension,

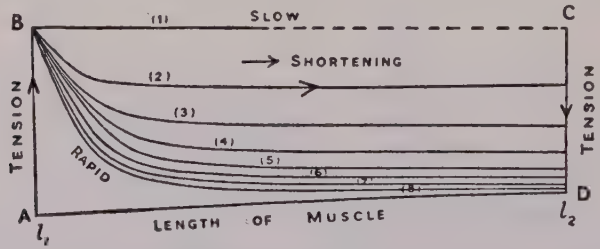


FIG. 54.—Superposed tension-length tracings of muscle, allowed to shorten at increasing speeds from (1) to (8). The speeds of shortening in arbitrary units were :—

(1) 0.083.	(2) 0.59.	(3) 1.31.	(4) 2.27.
(5) 3.79.	(6) 5.0.	(7) 6.58.	(8) 10.85.

(Levin and Wyman.)

the writing-point on the end of the beam describes an arc along the line *a*, *b*, which is thus the co-ordinate for change of length : when the beam is fixed, the muscle executes an isometric contraction the tension of which is recorded by the isometric lever L, writing along the arc *c*, *d*, which is the co-ordinate for tension. In making a tracing, the movements of the writing-point are traced on a *stationary* plate of smoked glass G ; the beam is fixed by the electromagnet, the muscle stimulated by the tetanising current for a definite time, and the line *c*, *d*, is recorded : the tetanus being maintained, the electromagnet is now released, and the muscle allowed to shorten at a controllable rate to a determined length, thus writing the tension-length diagram.

The initial and final lengths of the muscle are determined by the stops SS ; movement of the lever allowing shortening (or causing stretching) of the muscle is effected by the pull,

upwards (or downwards), on the arm NA, of the heavy weight W, the rate of this movement being controlled by an oil dash-pot P and needle-valve V. The tension-length diagram will be inscribed by the writing-point, moving in the direction shown by the arrow in Figs. 52, 53 from the point of tension reached at the prescribed initial length to a final variable tension at the prescribed final length, and the work

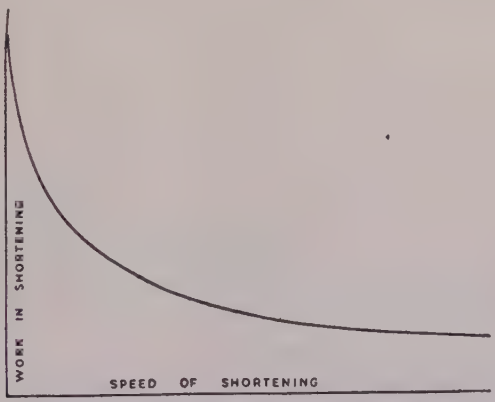


Fig. 55.—Relation between work done and speed of shortening. (A. V. Hill.)

done by the muscle in the shortening (or on it in lengthening) will be given by the area shaded in the figures (Figs. 52, 53). Curves made in this manner show that within limits the more slowly the muscle is allowed to shorten, the greater the work it does : on the other hand, the more quickly it is stretched, the more work must be done to stretch it.

A series of superposed shortening curves for tetanised muscle is shown in Fig. 54.

When the work (area of the tension-length curves) is plotted against the speed of shortening, it is quite clear that the work done by tetanised muscle is greater the slower the rate of shortening (Fig. 55).

The Viscosity of the Muscle Substance.—The substance of the muscle fibre possesses considerable viscosity, which plays

an important part in modifying the contraction of the muscle, and also gives to the tissue such definite properties as after-extension. A muscle when suddenly loaded at first extends quickly by reason of its elasticity, and then more slowly, because it is viscous.

The effect of viscosity is most clearly shown in contraction. When the muscle is stimulated, there is a liberation of elastic potential energy which tends to cause contraction. As soon as the muscle begins actually to shorten its movement is opposed because of the resistance offered by the viscous properties of the fibres, and some of the potential energy liberated by the contraction is used up in overcoming this resistance; the greater the speed of shortening, the larger the proportion of the energy of contraction wasted in this way. To take a mechanical analogy, considerable energy is required to stir a viscous liquid, such as treacle, as compared with that needed to stir a less viscous one, like water; the faster the treacle is stirred, the greater the energy lost in overcoming its viscous resistance.

We have already seen that when the load on a muscle is too great it shortens too slowly or (in the isometric contraction) not at all, and the energy which is set free on contraction, and not utilised for work, is converted into heat during relaxation. If, on the other hand, the load is too light, it moves too rapidly, and loses much of its energy in overcoming viscosity. In the maximum work devices which have been discussed above the load is variable, and at each stage of the contraction is just what the muscle can move, while, by varying the equivalent mass, the rate of shortening can be so adjusted that the shortening is just completed before relaxation begins. But the loss due to viscosity is necessarily associated with the act of shortening, and cannot be eliminated by the use of this apparatus. It can, however, be much diminished by dealing with prolonged contractions, and theoretically would vanish when the shortening took place with infinite slowness. Under even the most favourable

circumstances, therefore, the realisable work, though it may with increasingly slow contractions approach (asymptotically) nearer and nearer to the theoretical maximum work, will never actually reach this value.

As an illustration of this principle we may consider the results of an investigation by A. V. Hill. The calculations are complicated, and need not be referred to here, but ultimately led to the construction of the graph shown in

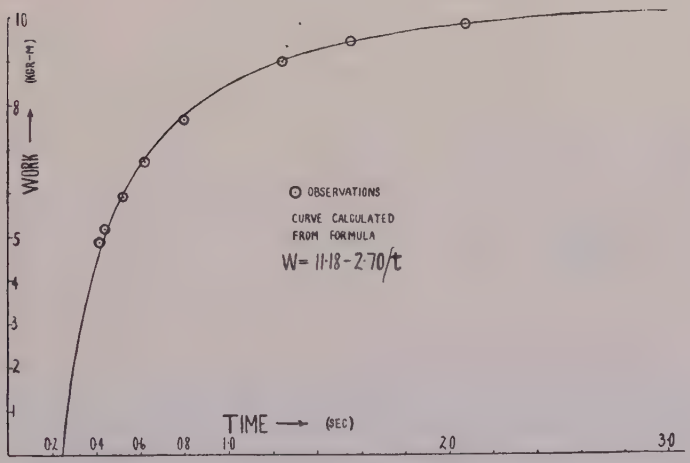


FIG. 56.—Relation between maximum realisable work and duration of contraction for human arm muscles. (Hill, *Journal of Physiology*.)

Fig. 56. The contraction studied was that of the arm muscles, using the inertia flywheel which has been described above. It is seen that the realisable work, shown on the ordinates, increases with the duration of the contraction, as given on the abscissæ, and ultimately reaches a fairly steady level when W = about 10 kg.M. Calculations showed that the theoretical maximum or " reversible " work was 11·18 kg.M., which is the work that would be realisable under these conditions when the contraction took place infinitely slowly.

Referring to the fact stated above (p. 169) that $W_o = Tl/6$,

it can be calculated that the tension which would be developed on isometric contraction by the biceps brachialis is about half a ton.

Mathematical treatment of results of this kind shows that

$$W = W_o (1 - k/t),$$

where W and W_o are the realisable and maximal theoretical work, t the time in seconds taken in the contraction, and k a constant which depends upon the properties of the muscle substance, $\frac{1}{k}$ being the greatest possible speed of shortening of the unloaded muscle. It is sometimes more useful to take, instead of t, the velocity of contraction, v, which $= \frac{1}{t}$, and then $W = W_o (1 - kv)$. The value of k for the human arm muscles employed was about 0·24, so that the equation for this instance becomes

$$W = 11{\cdot}18 - 2{\cdot}7/t,$$

or,

$$W = 11{\cdot}18 - 2{\cdot}7v.$$

The energy lost in overcoming viscosity is $(W_o - W)$, and the proportion of energy lost in this manner increases with increasing speed of shortening, according to the formula

$$\frac{(W_o - W)}{W_o} = \frac{k}{t} = kv,$$

that is to say, it varies directly with the speed of the movement.

In this formula k depends on the properties of the muscle, but not on its mass. It was also shown that at every stage of contraction the force exerted by the muscle was greater the less the rate of movement, and *vice versâ*.

A further investigation of the viscous properties of muscle was made on frog's muscles by Gasser and Hill. In their experiments a muscle, M (Fig. 57), fixed at A, was tetanised

and allowed to contract isometrically against the spring lever L, so that at first there was no shortening; then after a given interval, the tetanic contraction being still maintained, the end of the muscle fixed to A was suddenly released, the fixed point moving from A down to B at a certain prearranged distance towards the isometric lever. This arrangement allowed the muscle first to develop its maximal tension, and then to shorten, the rate at which it shortened being proportional to the distance from A to B.

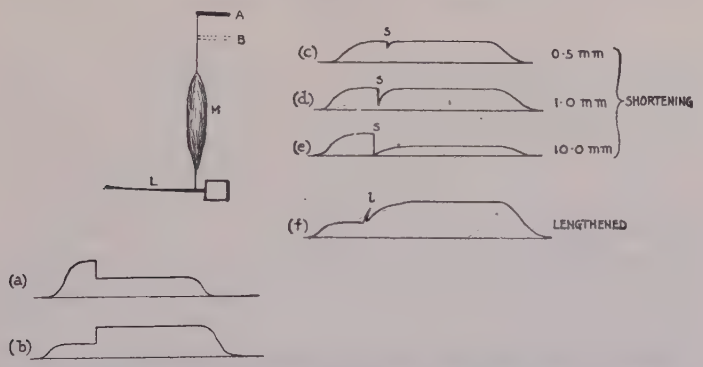


FIG. 57.—Diagram to illustrate the effect of sudden release or sudden stretching of a muscle during isometric tetanic contraction. (After Gasser and Hill.)

Only the principle of the experiments is illustrated in Fig. 57, which shows the arrangement in diagrammatic form. For fuller details the original paper must be consulted. In other experiments the muscle while contracted was suddenly pulled upwards and so stretched over a given distance.

If the muscle behaved merely as an elastic structure, then on releasing the muscle after the maximum tension had been reached so that it could shorten a definite distance, from A to B, the tension would merely drop to a level corresponding to the reduced length, as in Fig. 57 (*a*); conversely, if the muscle, after having set up its tension, were suddenly pulled from B to A, there should be merely an

instantaneous rise in the recorded tension (Fig. 57, (*b*)). Neither of these things actually happened, for even when the muscle was only allowed to shorten half a millimetre (and still more conspicuously when the shortening permitted was greater) the tension first fell considerably lower (Fig. 57, (*c*), (*d*) and (*e*)), even to zero, and then *slowly* rose to the proper level. Similarly, lengthening the muscle led, after a preliminary rise, to a slow rise to the final plateau (Fig. 57, (*f*)). This sluggishness of the contracted muscle in shortening or lengthening was attributed to the viscosity of its substance.

Increase of Viscosity on Contraction.—Comparison of the resting with the excited muscle indicates that the contracted muscle is not only viscous, but is much more so than the resting muscle. Changes in the viscosity of the cytoplasm of other cells as a result of stimulation have been demonstrated by Bayliss. The cytoplasm of the amœba contains many granules in active Brownian movement, and hence must be liquid: on stimulation the movement instantly ceases, suggesting that the cytoplasm has temporarily set to a gel state.

Gasser and Hill put this to the test by another method. If a steel spring is set vibrating, its vibrations die down quite slowly, according to a definite law of decrement. If a muscle is attached to the spring, so that each double vibration of the latter has first to stretch and then to release the muscle, the rate at which the vibrations are damped down is accelerated owing to the energy used up in overcoming the viscous resistance of the tissue. They found that this damping effect was much greater when the muscle was in the contracted than when it was in the relaxed state, and calculated that the damping coefficient was increased about sixteen times by contraction. There was also during the period of excitation an increased rate of vibration of the whole system, which means that the spring *plus* excited muscle behaved as a stronger spring than the spring *plus* relaxed muscle. In other words, the elasticity of the con-

tracted muscle, as well as its viscosity, was increased as a result of contraction.

As a result of such investigations, it is concluded that the tension actually exerted by a muscle during an isometric contraction does not represent the real fundamental mechanical response of the muscle, but lags for some time behind the fundamental mechanical response, because of the frictional effect of the high viscosity of the muscle substance.

Muscle as a Viscous-Elastic System.—Further information regarding the physical properties of muscle has been obtained by the use of the ergometer by Levin and Wyman, and has led them to the conclusion that muscle contains a two-part viscous-elastic system, of which one elastic part is free and the other damped. They succeeded in constructing a viscous elastic model of the type shown in Fig. 58. In this model the elastic part is represented by steel springs, while the viscous elastic part is imitated by steel springs, the movement of which is damped by baffle-plates working in an oil bath. Such a model closely imitates the reactions of muscle to release or stretching. For instance, if, as in Gasser and Hill's experiments on muscle, the stretched chain of springs be suddenly released, the tension will fall sharply because the springs A will shorten instantly, but after an interval, the springs B, sluggishly moving owing to their being damped, will also shorten, and will now again exert a pull on A, and so on the whole chain. Contraction in such a model we may regard as imitated by a sudden shorten-

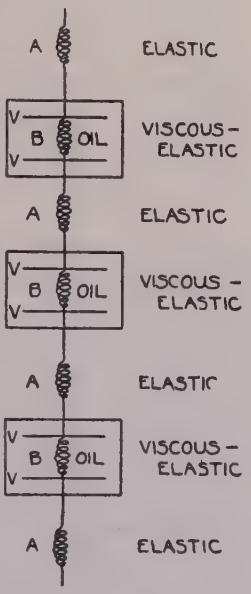


FIG. 58.—Diagram to illustrate the conception of a viscous elastic system. (A. V. Hill.)

ing of the B springs and an increase in the viscosity of the oil, A not being directly affected. Hence the slow rise of tension. When we say viscosity, we do not mean actual viscosity, so much as the sum of all those processes which resist the change of form. The apparent viscosities of the muscles of different animals appear to vary enormously according to their functions.

The Effect of Initial Extension and of Temperature Change.—In all muscle, extension of the fibre increases the energy (isometric tension) which is set free on contraction. Doi has recently shown that the maximal realisable work of the muscle is also increased by initial extension of its fibres. Beyond a certain optimal length further extension of the muscle leads to a reduction of the energy of contraction (Fig. 61, p. 192).

Rise of temperature reduces the tension set up on contraction and also the maximum realisable work (Doi); also, the rate at which the tension is developed during a contraction is affected by rise of temperature, as shown by Hartree and Hill. It was found that the suddenness of the isometric contraction was increased by rise of temperature, a rise of 10° increasing the rate of tension development about 2·5 times. Similarly, the rate of relaxation has a temperature coefficient of about 3·6, and the conclusion drawn from these observations is that chemical reactions form the basis of the processes of both contraction and relaxation, since the temperature coefficient of chemical reactions is of the same order of magnitude.

Relations to Stimuli.—After the application of an effective stimulus to a muscle its power of response to a second stimulus is abolished for a very short interval of time (absolute refractory period), which, according to the work of Adrian, is of the order of 0·003 second for frog's sartorius. Afterwards the excitability returns, and has reached the normal by about 0·02 second after the excitation. The recovery process as regards excitability has, according to

Adrian, a temperature coefficient of about 4. After recovery of excitability the tissue, according to Hartree and Hill, passes into a phase of super-normal excitability, in which the isometric tension shows an increase, which soon subsides to the normal.

The Mechanical Aspect of the Isotonic Contraction.—We have assumed thus far, as stated on p. 169, that, whether the muscle is allowed to shorten or not when it contracts, the potential energy (W_o) which is set free by contraction is the same, and may be measured by the tension set up isometrically, being, in fact, equal to $Tl/6$. Assuming this to be the sum total of energy available, we have seen that this potential energy, viewed as elastic potential energy, may be more or less completely converted into work when shortening is allowed to take place.

We have now to correct this assumption, in view of the fact that Fenn has shown that when the muscle is allowed to shorten the total energy at its disposal is greater than that obtainable on isometric contraction. In other words, it is incorrect to regard the theoretical maximum work, as derived from the stress-strain diagram or isometric contraction, as being all that the *shortening* muscle has at its disposal. It has an extra amount of energy, set free as the shortening proceeds, which enables it to shorten further and do more work than it otherwise would. To quote Fenn's own words: "The old and new theories of the nature of muscular activity in the performance of work may be exemplified by the following two methods of lifting a weight: (*a*) work may be done against a spring, which may then be allowed to shorten and lift the weight; (*b*) the weight can be raised directly by means of a chain and windlass. In the latter method every link of the chain which is wound up involves the expenditure of so much energy at the moment of winding. The chain may be under great tension, but, being inextensible, it possesses no potential energy. From the point of view of the energy exchange the shortening of a

muscle appears to be analogous to the windlass and chain rather than to the elastic band or spring, although it may partake to a limited extent of the characteristics of the latter."

We might perhaps thus compare the muscle lifting a weight to the winding up of a load by a stretched indiarubber cord wound on a windlass.

This view of the nature of muscular action is similar to that held long ago by Fick, but hitherto not capable of proper investigation owing to the technical difficulties.

The problem of the energy liberation in muscle, which at first appeared capable of solution along simple physical lines, such as the construction of the stress-strain diagram, is thus shown to be more complicated than was at first supposed ; to the energy liberated on isometric contraction there is added an extra amount of energy as the muscle shortens. The use of the various inertia devices for finding the maximum realisable work is also modified, for the work realisable should now be compared, not with the energy available under isometric conditions, but with the larger amount of energy made available under isotonic conditions of contraction. At present there seems to be no manner of predicting this amount or of comparing the realisable with the theoretical work in shortening except by determinations of heat production.

2. The Heat Production of Muscle

The heat production of muscle is measured by the use of thermopiles, which have been brought to a remarkable pitch of perfection by A. V. Hill. For certain purposes it is also possible to use a calorimeter made from a thermos flask, and to estimate the rise of temperature of water or Ringer's solution within it when muscles are kept in the calorimeter over a given rather long period. For the investigation of the time relationships of heat production and most other

purposes, however, the thermopile is what is employed. By suitable calibration the absolute heat production may also be determined by means of the thermopile.

The principle of the thermopile is so familiar that little reference to it need be made here. A thermopile such as is used by A. V. Hill is shown in Fig. 59. The muscle is held in the clamp, and lies on the junctions, made of suitable metal combinations, *e.g.*, gold and nickel, copper and constantan, etc., and with a suitable number of thermo-electric junctions changes of temperature of the order of $2{\cdot}5 \times 10^{-8}$ °C. can be observed by measurement with a sensitive galvanometer of the current produced. The pattern of thermopile must be adapted to the kind of work which is to be done with it. If required, the movements of the galvanometer may be recorded photographically, as was done by Hill and Hartree, and the time relations of the heat production can then be deduced for short tetani when due allowance has been made for the lag introduced by the transference of heat from the muscle to the thermopile.

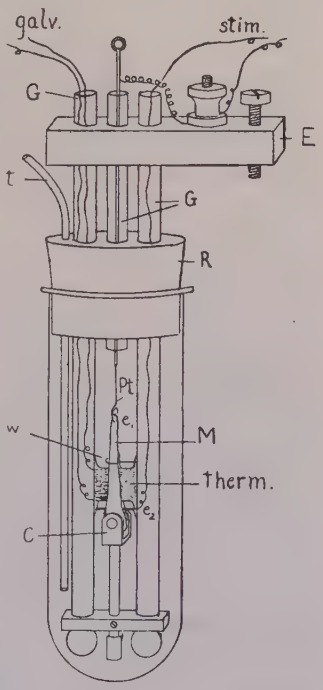


FIG. 59.—One type of arrangement of Hill's thermopile. (After Fenn.)

The value of a study of the heat production of muscle is that it enables us, after suitable calibration of the apparatus, to find the total energy exchanges over quite short intervals of time, and to compare these with the mechanical performance of the muscle under different conditions.

The Thermo-elastic Properties of Muscle.—It can be predicted on thermodynamical principles that muscle when passively stretched will show a temperature change, just as other substances do when they are stretched. Muscle when warmed (unlike most substances) shortens, and the prediction would be that the stretching of muscle should lead to a rise of temperature, and its shortening to a fall. This was found, on experiment by Hartree and Hill, to be what actually happened, though the result was complicated by the effect of viscous friction, which in both cases produced heat. Hence, on stretching the muscle, there is a liberation

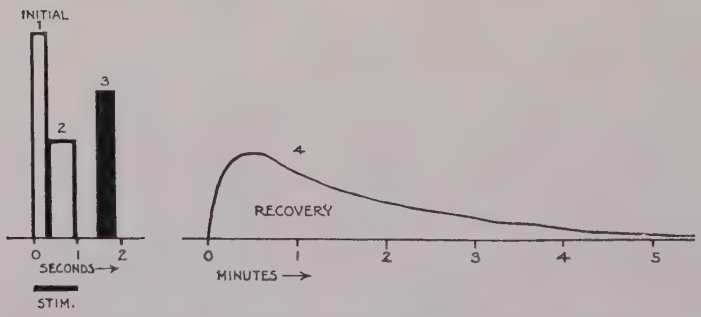


FIG. 60.—Diagrammatic representation of the stages of heat production of muscle in contraction and recovery.

of heat which is due partly to viscous effects, partly to the thermodynamic effect, while on releasing it there is first a fall of temperature, due to the thermodynamic effect of the shortening, followed by a rise of temperature due to viscous effects. These consequences of extension or shortening complicate the results in experiments where the muscle is allowed to shorten. The simplest results are, therefore, those obtained by muscles which contract isometrically, thus excluding these effects.

The Heat Production on Isometric Contraction.—When a muscle, under isometric conditions, is made to perform a short tetanic contraction, A. V. Hill and his collaborators

find that the heat production is not continuous, but appears to be liberated in four phases: (1) "initial" heat production which accompanies the *onset* of the contraction; (2) further "initial" heat production during the *maintenance* of the contraction; (3) more "initial" heat produced during *relaxation;* (4) a *prolonged* heat production during the *recovery* process, which follows the relaxation. These relationships are shown diagrammatically in Fig. 60. By "*initial heat*" is understood that included in (1), (2) and (3), and by "*recovery heat*" production that under (4). Now it has been shown that the initial heat production is the same whether the muscle is made to contract in oxygen or in nitrogen, or if its oxidative processes are checked by placing it in dilute cyanide solution. It seems clear, then, that the initial heat production corresponds with the break-down processes or chemical changes which bring about and maintain contraction. Peters has shown that 1 gram of muscle, when stimulated to complete fatigue in absence of oxygen, produces about 0·9 calorie. Knowing from this determination, and also from the determinations of Meyerhof and Suranyi, of the amount of lactic acid produced, it is possible to calculate that in the production of 1 gram of lactic acid in anærobic contraction about 385 calories are set free. From such data as these, and according to those of heat production based on determinations by Hartree and Hill, the following balance-sheet may be drawn up for the heat production of frog's muscle in isometric twitches:—

Phase.	Cals. per gram. Lactic Acid.	Relative Amount. (Initial = 1.)
In absence of oxygen:		
Contraction and relaxation (=initial heat)	385	1·0
In oxygen:		
Contraction	255	
Relaxation	130	385	1·0
Oxidative recovery	410	1·07
Total in oxygen	795	2·07

It will be seen that, although the initial heat production is the same in presence as in absence of oxygen, there is in oxygen a large " recovery " heat production following the contraction, and about equal to the " initial " heat.

When tetani are studied, the results are somewhat different, because the heat production for a given tension is greater. This is for two reasons : first, because energy is needed for maintenance of the tension, and, secondly, because after a tetanus there is always some delayed heat production even under anaerobic conditions : this delayed anaerobic heat is at present unexplained. It is usually about 0·25 of the initial heat, but varies widely.

These results, when compared with the chemical investigations of Meyerhof and others, indicate that the recovery heat production is due to oxidative changes associated with removal of lactic acid and re-formation of glycogen. For each gram of lactic acid formed in absence of oxygen in the initial stages of the contraction process, about 0·79 gram is restored to glycogen again in the course of recovery, the remainder, 0·21 gram, being burned (*v.* p. 141). The net result of exhaustion and recovery is, then, that 0·21 gram of glycogen is burned. Since Slater finds that 1 gram glycogen, on combustion in dilute solution, yields 3,836 calories, the heat of combustion of 0·21 gram glycogen would yield 806 calories, thus agreeing with that found by myothermic observations (actually 795 calories, = 0·207 grams glycogen).

It is possible, however, to make a more complete analysis of the phenomenon than this, and so to trace the energy exchanges through various stages. For this purpose we have the following data for anaerobic contraction :—

Heat of combustion of hydrated glycogen per gram . . .	=	3,836	calories
Heat of combustion of 1 gram lactic acid in solution	=	3,601	,,
Heat of neutralisation of 1 gram lactic acid by salts	=	19	,,
Heat of neutralisation of 1 gram lactic acid by proteins	=	140	,,

The actual heat of neutralisation has been estimated by Stella as 105 calories, viz., about $\frac{3}{4}$ of the heat of neutralisation by

protein, which appears to be the method that is chiefly involved. The remainder of the 385 calories, viz. 45 calories per gram lactic acid, is not yet accounted for. The molecule of hydrated glycogen is $C_6H_{10}O_5 . H_2O$, and a gram of this (= 0·9 gram of anhydrous glycogen) forms a gram of lactic acid.

Hence we have for each gram lactic acid—

1 gram hydrated glycogen → 1 gram lactic acid = 3,836 − 3,601 calories	=	235 calories
Neutralisation . .	=	105 ,,
Unexplained	=	45 ,,
Total	=	385 ,,

When oxidative recovery is allowed to take place, we should have, assuming the heat of combustion of 1 gram of lactate in the muscle is 3,601 − 105 = 3,496 calories—

(1) 0·21 gram lactic acid → $CO_2 + H_2O$ + 734 calories

and since

Heat of combustion of 0·79 gram glycogen	=	3,031 calories

and

Heat of combustion of 0·79 gram lactic acid as lactate	=	2,762 ,,
Difference	=	269 ,,

then we may write

(2) 0·79 gram lactic acid → 0·79 gram glycogen with absorption of 269 calories,

the net result of the oxidative recovery being therefore 734 − 269 = 465 calories, instead of about 410, as given above for the observed value.

In tetani, more heat, viz. 859 calories per gram lactic acid, is produced in the total cycle, and many of the data were obtained from tetani. In that case the heat of recovery per gram lactic acid would be 859 − 385 = 474 calories, which agrees better. The methods employed necessitate so many corrections and allowances that exact agreement between chemical and myothermic methods is obviously very difficult to reach.

It does not necessarily follow that the maximum mechanical work which a muscle might perform is the same as that which results from energy considerations derived from heats of combustion. It may, under certain conditions, be greater, and depends on the " free energy " of the changes, thermodynamically considered. Burk has calculated that the free energy of the

change from glycogen to lactic acid is probably some 50 to 100 per cent. greater than the corresponding heat of reaction ; under anærobic conditions, therefore, the muscle might be able to perform more mechanical work than corresponded with the calculated heats of reaction. For ærobic conditions the free energy is about equal to the heat of the reactions. The importance of the consideration of free energies was already emphasised by A. V. Hill in 1912 (*q.v.*).

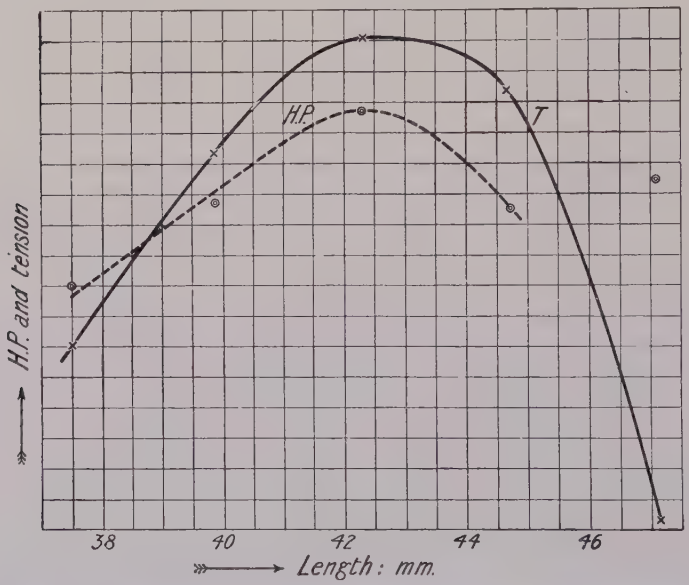


Fig. 61.—Relations of heat and tension production to initial length of muscle. (After Evans and Hill.)

As regards the rate of recovery heat production, it has been shown by Hartree and Hill that the greater the extent of the initial break-down, *i.e.* of the initial heat production, the more rapid the rate of the recovery process.

Relation of Heat and Tension produced.—The total initial heat formed in isometric contraction and relaxation is a measure of the total energy set free by the muscle in the act of its contraction, and, as has already been pointed out, the tension set up under isometric conditions bears a constant

relationship to the heat production, the ratio T*l*/H remaining constant at an average value of 6·16, under a variety of conditions. This ratio is a measure of the perfection with which the chemical potential energy is converted into mechanical potential energy, *i.e.*, of the efficiency of the muscle. Thus it is almost unaltered by temperature, which means to say that both the tension and the heat production are affected equally by alteration of temperature. The relation is, however, affected by the strength of the shocks used to stimulate, and also by the initial extension, being reduced by increase of either.

Increase of initial extension, as shown by Evans and Hill, causes a rise of the heat production, as well as of tension production, up to a certain maximum, beyond which there is a decline in both. (Fig. 61). The ratio T/H, however, declines with initial extension. The maxima of heat production and of isometric tension are reached when the muscle has been extended to 1·4 to 1·5 times its unloaded initial length. Curiously enough, increase of initial length retards the rate of *relaxation*.

The fact that T/H is diminished by increasing strength of stimuli, although both T and H are increased, is interesting. The heat production is increased more than the tension set up; that is to say, we cannot regard the "all or none" principle as strictly true, for if it were, then with an increase in the strength of the stimulus we should get an increased number of fibres contracting, and the same proportion of the energy utilised (H) should be converted into tension (T), so that T/H should remain constant. It must be that the character of the response, as well as the number of fibres responding, alters as the strength of the stimulus is increased. This is further shown by the interesting fact, for which there appears at present to be no adequate explanation, that a super-maximal stimulus causes a smaller heat production than a maximal one, though the mechanical effect of both is the same. The efficiency of the contraction produced

by super-maximal stimulation may for this reason be some 15 per cent. higher than that produced by the maximal shock.

The question whether the thermal response and the mechanical response of a muscle are necessarily connected must be answered in the affirmative. Earlier work had suggested that, by the use of suitable narcotics or by treatment of the muscle with water, the mechanical response could be eliminated while the heat production was in some measure retained, just as it was believed that the electric variations in the muscle were also retained under these conditions. Gasser and Hartree have reinvestigated the question, and have shown that, when proper measures are taken to detect small tension changes, the reduction of tension and of heat production go hand in hand, and extinction of the two takes place simultaneously. The tension, however, falls most rapidly; in other words, the efficiency of the contraction is considerably reduced as the damage of the tissue progresses. In extreme fatigue also, the ratio Tl/H falls greatly.

Energy Utilisation in Prolonged Contraction.—Hartree and Hill studied prolonged isometric contraction, and the effects upon it of alterations of temperature. The relation of the heat production to the duration of the contraction and to the temperature is shown in Fig. 62. It will be seen that when the muscle is tetanised for very short intervals of time (*e.g.*, for 0·01 second) the heat production is higher at the lower temperature, as shown in the small figure inset in Fig. 62. When the tetanic stimulation is applied for about 0·02 second the heat production is the same at all temperatures; while when the stimulus lasts for any time longer than this the rate of heat production is higher at the higher temperatures, and at all the temperatures soon takes the form of a straight line, which means that heat is being produced at a steady rate as soon as the preliminary period of rise has been passed. If we compare the slopes of the curves at different temperatures, we find that the effect

of a rise of 10° C. is to increase the steady rate of heat production about 2·8 times; this temperature coefficient is held to imply that some chemical phenomenon lies at the root of the steady production of heat in maintaining the contraction. Perhaps it means that there is a steady rate

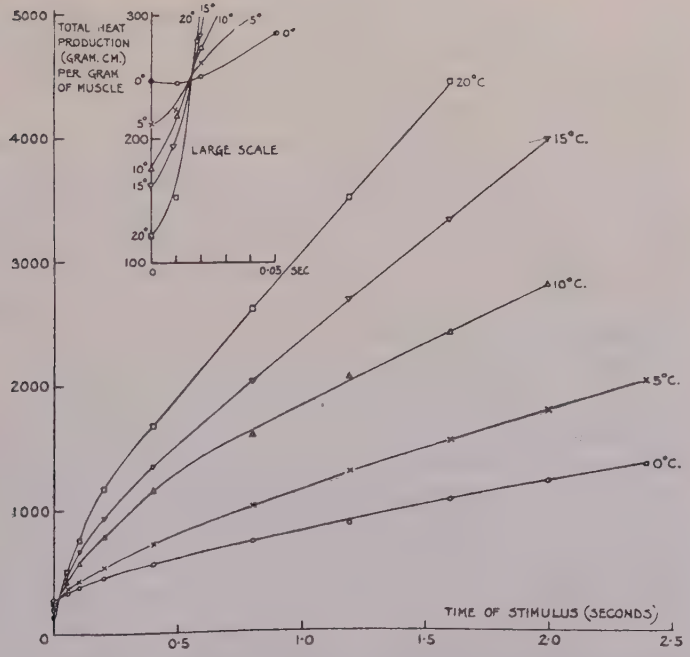


FIG. 62.—Rate of heat production in sustained isometric contractions of various durations. (After Hartree and Hill, *Journ. of Physiol.*)

of break-down of the lactic acid precursor. If the ratio H/T*l* is plotted against the duration of the stimulus, we find that, whereas in the isometric *twitch* the efficiency is high (and the value of the ratio H/6T*l* expresses its efficiency in producing potential energy), yet for the *maintenance of the tension* the efficiency in isometric contraction is quite low. This low efficiency of the protracted contraction is due to

the fact that, as stated on p. 189, energy is utilised, and converted subsequently into heat, in the upkeep of the tension. Mathematical treatment of the results shows that the total energy utilised (or heat production)

$$H = aTl\,(1 + bt),$$

where t is the duration of the stimulus and a and b are constants, a being unaffected by temperature and b having a temperature coefficient about equal to that of a chemical reaction. Now aTl is, as shown by the consideration of the stress-strain diagram and of the facts already dealt with on p. 193, a quantity which is proportional to the potential energy developed, and in the mere twitch represents all the energy utilised. But the quantity $bt(aTl)$ represents the energy utilised in keeping up the state of tension aTl for the time t. The constant b thus represents the actual waste of energy in keeping up the contraction.

The cost of maintaining contractions varies very much in different muscles, and especially in different animals. It is much higher in the frog than in man, and hence the mechanical efficiency of movements in the frog are much lower than in man, because much energy must be lost, either through viscous resistance when the contractions are rapid, or through maintenance when they are prolonged.

Application to Human Muscle.—These facts apply, with certain modifications, to human muscles.

The first modification results from the fact that most of the data dealt with above relate to maximal contractions, in which all the fibres are involved. This is true also for the experiments on the shortening of human muscles, using the inertia device. In conditions of daily life, however, it more often happens that only certain of the fibres are utilised in a contraction ; but since each of these fibres contracts on the " all-or-none " principle, we may assume that the laws relating realisable to theoretical maximal work for a whole muscle in maximal contraction also hold good for each fibre

of a muscle engaged in submaximal effort. Hence, if w_o be the " reversible " work of each fibre, W the work to be done, and n the number of fibres, which contract at the speed v, then

$$W = n\, w_o\, (1 - kv),$$

or, in other words, the number of fibres required to do the work W at the speed v will be

$$n = \frac{W}{w_o\,(1 - kv)} \quad ,$$

and it is evident that, as the speed becomes greater, more and more fibres must be utilised, until finally all of them are involved. The number of fibres utilised will thus depend not only on the work to be performed, but also on the speed with which it is to be done ; in other words, on the physiological effort involved. This, as everyone knows from personal experience, may be great, and correspondingly exhausting, either because the work to be done is great or because the speed at which it is performed is high.

Another essential modification consists in the consideration of the recovery process, as well as of the processes represented by the initial heat production. We have seen (p. 189) that the whole process of contraction and recovery with oxygen liberates twice as much heat as is set free in the anaerobic processes of contraction. Now we already know that we may, as a first approximation, consider the theoretical maximum work (W_o) as proportional to Tl, and hence to aTl. Hence we may say that the total energy (H) set free in contraction (and recovery) should be

$$H = 2\, W_o\, (1 + bt).$$

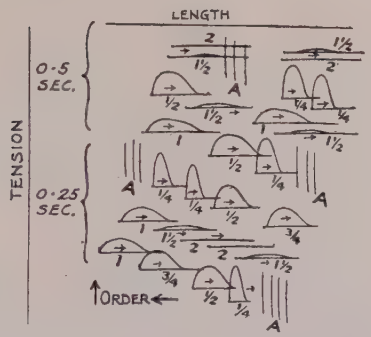
When the muscle shortens, the relations will, in the light of Fenn's work, no doubt be altered, but we cannot yet say to what extent.

Mechanical Efficiency of a Contraction.—We have already seen (p. 180) that the realisable work $W = W_o\,(1 - k/t)$.

The mechanical efficiency of a contraction is the fraction of the total energy expended which can be recovered as work ; *i.e.*, it is W/H.

The work done by a muscle can be increased by reducing the speed of shortening, because by that means the effect of internal resistance is reduced ; but, from the point of view of mechanical efficiency, since prolonged contractions require energy for maintenance, it is clear that the greatest efficiency will be obtained with contractions of relatively short duration : the point of optimum efficiency can be determined by the use of the Levin and Wyman ergometer, which allows the muscle to shorten at any desired speed and measures the maximum work which it was capable of performing at that speed. In a recent research, Hartree and Hill employed this instrument and also made simultaneous observations of the heat production accompanying each contraction of frog's sartorii. The total energy expended is the sum of heat produced and work done. Records of the tension-length curves of the muscles stimulated for 0·25 and 0·5 seconds, and allowed to shorten at various rates, are shown in Fig. 63, and the work done in Fig. 64.

It will be seen that the work done is greater with the more prolonged contractions, but that in both cases there is a particular speed of shortening at which a maximum amount of work is done. It was found that the total energy liberated varied with duration in a similar way to the work done, and the maximum efficiency was reached at about the point of maximum work. The maximum efficiency was found to be independent of the temperature and almost independent of the duration of the stimulus. The mean value, for the initial process only, was 26 per cent. : if the recovery process is taken into account the maximum efficiency would be only $12\frac{1}{2}$ per cent. This is so much less than in human muscles because maintenance of contractions is so costly in frog's muscle that the highest efficiencies are only obtained with very brief contractions ; hence, shortening must be rapid, or



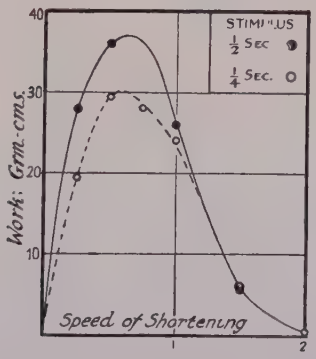


FIG. 63.—Upper fig. records with Levin-Wyman Ergometer, showing effect of speed of shortening on the work done in contractions of limited duration. Arrows show direction of shortening : numbers the speed of shortening in arbitrary units. A = isometric, *i.e.* shortening speed = 0.

FIG. 64.—Lower fig. results of the above tracing plotted as a graph, in which ordinates = work done (area of curves). Abscissæ = rates of shortening. (Hartree and Hill.)

else relaxation would set in. In man, maintenance is less costly and contractions can be so slow that viscosity effects are much reduced.

The approximations detailed in the last section enable us to form some idea of the mechanical efficiency of the human muscular contractions, for

$$\frac{W}{H} = \frac{W_o (1 - k/t)}{2 W_o (1 + bt)} = \frac{(1 - k/t)}{2 (1 + bt)}$$

The value of k can be found, by means which need not be discussed here, to be about 0·24 for the biceps brachialis of a healthy man, and it is then found that the *optimum duration* of the contraction

$$x = k \left[1 + \sqrt{1 + \frac{1}{klb}} \right];$$

from which the value of $b = 0{\cdot}2$.

When this value is taken, the efficiency of the maximal human biceps contraction is

$$\frac{W}{H} = \frac{\left(1 - \frac{0{\cdot}24}{x}\right)}{2 (1 + 0{\cdot}2\, x)}.$$

If the results of efficiency calculations determined in this way are plotted against the duration of the contraction (x), it is found that the efficiency at first rises steeply as the contractions become more prolonged, and for those particular muscles the maximum efficiency is obtained when the contraction takes about 1·4 seconds, after which it falls off slowly. The maximum efficiency obtained is about 0·26, *i.e.*, 26 per cent. It is of interest to note that efficiencies of this order (25 per cent.) were also obtained in my experiments on the heart, and that I likewise found that when the muscle shortened more quickly, as with the increase of output, the efficiency rose up to a limiting value and then fell off again. In fact, the maximum efficiencies obtained in all muscular performances are of much the same value, as is seen in the ergometer studies of Benedict and Cathcart on men using the bicycle ergometer, the energy utilisation being determined by measurement of the gaseous metabolism.

In experiments on man we may determine from the respiratory exchange either the gross or the net efficiency.

It is known that the number of calories of energy obtained from the utilisation of each litre of oxygen (which is of the order of 5 (large) Calories per litre) varies slightly with the respiratory quotient. It is, therefore, necessary also to determine the respiratory quotient, and then, by reference to the subjoined table, the calorific value of oxygen is obtained.

Calorific Value of Oxygen.

Respiratory Quotient.	Calories per litre Oxygen.	Respiratory Quotient.	Calories per litre Oxygen.
0·70	4·686	0·90	4·924
0·75	4·739	0·95	4·985
0·80	4·801	1·00	5·047
0·85	4·863		

When we determine the oxygen usage during the performance of an exactly measured amount of muscular work (W), we can from this calculate the total energy usage (H) by the use of the proper factor for the calorific value of oxygen. The results (W/H) thus obtained give us what is called the *gross efficiency*. If we deduct from the total oxygen usage an amount equal to that required by the resting body (h), we find the increment of oxygen, $H' = H - h$, used on account of the performance of the work, and calculations of W/H′ on this basis give us the *net efficiency*. It has

Maximum Gross and Net Efficiencies of Subjects in Respiration Experiments with the Bicycle Ergometer.
(From Benedict and Cathcart.)

Subject.	Gross Efficiency. Per cent.	Net Efficiency. Per cent.
E. P. C.	19·9	23·1
J. J. C.	17·8	20·4
H. L. H.	18·6	21·6
J. E. F.	19·8	22·7
K. H. A.	18·2	20·8
M. A. M.	21·2	25·2

been questioned whether the net efficiency is the more correct to take, though, of course, it gives the higher values of efficiency. A summarised table of net and gross efficiencies, according to Benedict and Cathcart's study of work on the bicycle ergometer, is given on p. 201.

This shows that the calculations made by Hill for the efficiency of the contraction of an individual muscle are of much the same order as those found in the determination of the net efficiency of the whole body when carrying out exactly determined amounts of work. Hill's calculations should be compared with the net efficiencies rather than with the gross, because not a very large fraction of the basal oxygen requirement of the body is made up by the respiration of really resting muscle. Further applications to human muscular contractions will be considered in the next chapter.

The Energy Production in Isotonic Contraction.—This account of the energy production of muscle would be incomplete without some further reference to the interesting suggestion which arises out of Fenn's work that when a muscle shortens in its contraction it can draw upon sources of energy which are not utilisable, or are not fully utilised, in the isometric contraction. If this work stands the test of further examination, then all the inferences made from the study of the isometric contraction will need to be considerably modified when the isotonic contraction is discussed. For example, the conclusions which have been drawn from the tension and heat production, and from the relation between them, will have to be adapted to the newly accepted conclusions from Fenn's work.

The first series of experiments carried out by Fenn consisted in determining by improved methods the heat production of frog's muscles on isometric and isotonic contractions. Some of his results are given in a simplified form below; they show that when the muscle shortens it not only does work, but also generates more heat than under isometric conditions.

(From Fenn, *Journal of Physiology*, 1923, **58**, p. 180.)

Mode of Contraction.	Weight lifted.	W = Work done, ergs × 104.	H = Heat Production, ergs × 10⁴.	Efficiency = W/H, per cent.
Isotonic.	50 grams.	1·26	9·3	13·5
,,	100 ,,	1·88	10·5	17·9
,,	200 ,,	1·90	11·5	16·5
,,	300 ,,	0·62	8·6	7·2
Isometric.	∞	0·0	7·3	0·0

A second series of experiments was then undertaken, in which, by the use of a special device, different weights were lifted always to the same extent. It was found that when the distance through which the weights were lifted was comparable with the normal amount of shortening in the frog's muscle *in situ*, the increase in the energy liberated and measured as heat was about 2·3 times the increase in the work done; *i.e.*, it was proportional to the tension under which the shortening took place.

In a third series the load was kept constant, while the degree of shortening was varied, and again there was found an increase of heat production above that found for the isometric contraction.

In the fourth series the inertia lever was used, so that at each stage of its shortening the muscle was pulling against a resistance which it could only just overcome, and the heat produced was measured. The results of such an experiment are summarised in slightly modified form on p. 204.

This table shows quite clearly that in the shortening process the muscle has considerably greater energy utilisation (shown in the last column) than it has in the isometric contraction. It appears from this and other experiments that the excess heat production is roughly proportional to the work which is done. This means that the muscle adapts its

energy expenditure to suit the amount of work which it has to do.

In a further study of the energy liberated when work is done, Hartree and Hill find that the Fenn effect is only obtained when the muscles are tetanised. They studied the relations between heat and work by means of the Levin and Wyman ergometer, together with appropriate myothermic arrangements, and found that in single twitches, the total energy set free is the same whether work be done or not: the heat of relaxation is diminished by an amount equal to

(After Fenn, *Journal of Physiology*, 1923, **58**, p. 191.)

Mode of Contraction.	Equiv. Mass, kg.	Work done, ergs × 10^4.	Total Heat, ergs × 10^4.	Excess Heat over Isometric, ergs × 10^4.
Isometric.	0	0	10·35	0·0
Isotonic.	0·36	2·17	13·11	2·76
,,	0·80	2·64	14·55	4·20
,,	1·42	2·88	15·28	4·93
,,	3·19	3·06	15·43	5·08
,,	5·69	2·81	14·95	4·60
,,	12·76	2·36	13·87	3·52

the work done. This is confirmation of the all-or-none principle in muscle.

In tetanic contraction, the work done is also at the expense of the relaxation heat; the excess heat production which appears under these conditions owing to work being done is seen as an increment in contraction heat. Work done by the muscle during the relaxation phase, *e.g.*, when it is allowed to shorten after the stimulus is over, has no effect on the energy set free. It is suggested that these results show that the work done and the heat of relaxation are both derived from the mechanical potential energy of the stimulated muscle. Hartree and Hill suggest that contraction is due to the increase of some unknown "intensity" factor, which is diminished by the performance of work—hence the irreversible relaxation-heat is reduced. This intensity factor can be kept constant, while work is being done, only by continued stimulation—hence the increased heat production during a tetanus. If this view is accepted, then, as they point out, the

tension-length curve does not give the theoretical maximum work except for slow shortening in tetani. Probably the relaxation heat gives a truer measure of the potential energy; it is about half the area of the tension-length diagram.

The Effect of Chemical Substances on Heat Production.—The influence of chemical substances on the heat production of muscle is interesting because by the study of such effects light is thrown on the mechanism of contraction and recovery. We will glance at some examples of such investigations.

The influence of hydrogen ion concentration is of interest because the lactic acid which is liberated in the initial stages might conceivably raise the hydrogen ion concentration of the muscle fibre. Roaf, indeed, carried out experiments which were thought at the time to have shown that the reaction of the muscle became more acid in the course of its contraction. These experiments have since been criticised, and at present it cannot be said definitely whether or not the muscle becomes appreciably acid in the course of an ordinary twitch. The indications are that there is no very pronounced increase in the acidity, because the muscle substance is endowed with a plentiful supply of buffers, such as phosphates and proteins, which keep the hydrogen ion concentration constant for a considerable time. But when contraction has been continued to the point of fatigue, there can be no doubt that the accumulation of lactic acid is so considerable that the muscle substance does become definitely less alkaline. Now Hartree and Hill have shown that, starting from neutrality, an increase in the hydrogen ion concentration of the fluid in which the muscle is immersed greatly retards the speed of the recovery process, as judged by the rate of heat production in the stages which follow contraction. But between the limits of *p*H 10·2 and neutrality, *i.e.*, on the alkaline side of neutrality, reduction of the alkalinity does not affect the speed of the recovery process.

The prolonged contraction in response to a single stimulus which is seen in a muscle previously treated with veratrine has been studied by Hartree and Hill. It was found that the

tension and heat production on isometric contraction were similar to those which obtained in prolonged tetanic contractions, and the inference was that veratrine produced its effect not by holding the process of relaxation in abeyance, but by prolonging the break-down changes produced by stimulation.

Hartree and Hill found that when the muscle was treated with caffeine, which is known to produce a stimulating effect on it, there was a steady and long-continued liberation of heat, which was continued whether the muscle was in presence of oxygen or not, and may be regarded as caused by a continual break-down of the lactic acid precursor. The disappearance of glycogen and also the rate of accumulation of lactic acid are accelerated in striated muscle by caffeine.

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CHAPTER VIII

APPLICATIONS TO THE PHYSIOLOGY OF MUSCULAR EXERCISE IN MAN

Lactic Acid in Blood.—There are no *à priori* reasons for supposing that the phenomenon of muscular contraction is essentially different in man and in the isolated muscles of the frog. But the conditions under which the contractions take place are decidedly different in various respects. Thus the muscles in man are at a higher temperature, are provided with a blood supply, and are under voluntary control from the central nervous system.

When the muscles contract, lactic acid is produced; the more frequent or powerful the contractions, the more rapid the rate of formation of the lactic acid. Now lactic acid has been detected in the blood of normal resting men by many investigators even after they have breathed oxygen for some time. The amount of resting lactic acid may be taken as of the order of 5 to 20 mg. lactic acid per 100 c.c. blood. The explanation of its presence may be that even in the body at rest there is a certain amount of muscular activity, which implies a constant rate of production of lactic acid, some of which diffuses out into the blood and is only slowly removed—where is uncertain.

Macleod and Knapp demonstrated that when the blood was made more alkaline, lactic acid was present in increased amount in the circulating blood. Now Anrep and Cannan have shown with the heart-lung preparation that the lactic acid of the blood is lowered when carbon dioxide is added to the blood and raised when it is removed. It appears from this that when the blood becomes less alkaline lactic

acid destruction is accelerated, while when it becomes more alkaline lactic acid accumulates. This suggests that the very accumulation of lactic acid in the blood, which would make the blood less alkaline, will bring about an accelerated rate of lactic acid removal, and so lead to a state of balance at a lower level than would otherwise be obtained. The following experiment illustrates the magnitude of the effect :–

Effect of Carbon Dioxide on Lactic Acid of Blood in Heart-lung Preparation (Anrep and Cannan).

Time.	Treatment, lungs ventilated with	Lactic Acid of Blood, mg. per 100 c.c.	CO_2 Content of Blood, volumes per cent.	*p*H of Blood.
1.20	Air . . .	85	12	7·68
2.30	Air + 6·8 per cent. CO_2 for 1 hour . .	52	45	7·32
3.30	Do., 2 hours . .	32	51	7·34
4.30	Air + 9·2 per cent. CO_2 for 55 minutes .	21	66	7·2
5.30	Air only, 1 hour .	59	18	7·8

In confirmation of these experiments, Long found that the lactic acid in the blood of resting men fell to the vanishing point in about half an hour when the inspired air had about 10 per cent. of carbon dioxide added to it.

During muscular activity lactic acid is certainly being produced at a greatly accelerated rate, and, in spite of the mechanism just described, tends to accumulate in the blood, and even to be excreted in small amount in the urine, as has been shown by Ryffel, by Campbell, Douglas and Hobson, and others. The rate of accumulation of lactic acid in the blood depends on the severity of the exercise ; if this is so severe that the lactic acid is being produced faster than it can be removed from the muscles and blood, then the amount of acid in the blood can reach a relatively high figure, but if the

oxidative processes in the muscle can cope with the lactic acid and remove it as fast as it is formed, the escape into the blood will be small. These facts are shown by the following table, taken from some experiments by Hill, Long and Lupton :—

Lactic Acid Accumulation in Blood.

Lactic Acid in Blood, mg. per cent.		Type of Exercise.
At Rest.	After Exercise.	
20·9	36·6	Walking 3·5 miles per hour.
21·4	58·9	Walking 4·1 miles per hour.
20·0	86·0	Standing running at 300 steps per minute for 4 minutes (breathing pure oxygen).
8·5	204·0	Standing running at 239 steps per minute for 9½ minutes.

The first two examples are for moderate amounts of exercise, the last two for severe exertion.

Observations of the alveolar air after exercise by J. B. S Haldane and Quastel indicate that only a small fraction of the total lactic acid formed in the muscles finds its way into the blood. Thus the alveolar CO_2 pressure was only lowered at most by 8·6 mm. Hg, which would correspond to neutralisation of bicarbonate by about 2·8 grams of lactic acid in the blood of the whole body. If this is so, the muscle must therefore retain most of its lactic acid.

The Oxygen Intake

The Effects of Violent Exercise on the Oxygen Intake.—It would be expected that as a result of really violent muscular exercise there would be a considerable accumulation of lactic acid in the muscles, and to some extent in the blood, which

would be oxidised away *after* the exercise was over. When the exercise was less severe, it would be anticipated that the accumulation of lactic acid would be much smaller, because most of it would be oxidised away during the exercise, the formation of fresh lactic acid just balancing the oxidation of that previously produced, so that a steady state would be set up in which the concentration of lactic acid in the muscle was quite low. This steady state would be reached because as the lactic acid tended to accumulate the oxidative reactions by which it is removed would be accelerated, just as we have seen to happen in the blood.

These expectations were justified by the work of Campbell, Douglas and Hobson, who found that the oxygen intake is increased not only during the time that the exercise is in progress, but also for some time after its cessation ; the more violent the exercise has been, the more prolonged is the increased oxygen usage after it has been completed. This prolonged augmented oxygen usage can be explained as due in part to the oxidation, in the recovery process, of lactic acid which has been produced during the exercise. The exercise in their experiments was performed on the bicycle ergometer. It was found that after completion of the exercise the oxygen intake fell quite rapidly to nearly resting values.

The increased gaseous exchanges of the body following more violent exercise, such as that of running, have been recently studied from the view-point of lactic acid removal by A. V. Hill and his co-workers, who have shown that a study of the oxygen intake and CO_2 production during the recovery from exercise gives results which agree with expectations based on the study of isolated muscles. These investigations were made by a technique in which the Douglas bag method was used for the determination of the respiratory exchange and in which an attempt was made (*a*) to determine the aggregate excess oxygen use during the entire recovery period and (*b*) by the use of short periods of collection to

obtain an idea of the intensity of oxygen usage at different intervals.

It should be remembered that in running the mechanical energy is being converted into kinetic energy, which varies as the square of the velocity. Hence, in running at velocity v, energy must be provided at a rate proportional to v^2 per yard covered : *i.e.*, the work done is $= av^2$, where a is a constant, and since we know that W is also $= nw_o(1 - kv)$, therefore

$$nw_o = \frac{av^2}{1 - kv} \text{ per yard.}$$

But since the velocity per second is proportional to v, then the energy required per second will be proportional to $\frac{v^3}{1 - kv}$. It has been shown by direct experiment by Sargent that the energy expenditure in a 120 yards sprint by an experienced runner does actually vary as the 2·8th power of the speed, while the oxygen requirement in unit time increases as the 3·8th power of the speed. The energy expenditure involved in running 120 yards in 13 seconds amounts to nearly 14 horse-power.

Let us consider the chain of events which may be considered to happen in the muscles of the body during a bout of very severe exercise, such as a sprint or in "standing running," *i.e.*, a very rapid mark-time movement of the legs, which are moved as in running for, say, thirty seconds. During these violent muscular contractions lactic acid is being rapidly formed, and such of it as cannot be removed by oxidation at the time must accumulate in the muscles. Actually, under such conditions of maximal effort, very little of it can be so oxidised. It will, therefore, continue to accumulate in the muscles until its concentration there is high enough to limit or entirely stop their further action ; with maximal efforts this happens in about half a minute.

During the period of exercise the amount of oxygen which

has been taken in by the subject is found to be by no means large compared with the amount that would be required to prevent the accumulation of lactic acid. Let us suppose that during this half-minute the subject has used 2 litres of oxygen from the inspired air, and that further, by completely utilising all the oxygen which was present in his blood at the beginning of the exercise (which is unlikely), he has obtained a further 800 c.c., total 2·8 litres. These are outside figures, and actually much smaller figures would be found. This total available oxygen is relatively so low, not because he does not need more, but because he cannot get it faster ; the limiting factor is the heart, which cannot pump blood quickly enough to supply to the tissues the oxygen which will enable them to oxidise the lactic acid as fast as it is being formed.

Now let us follow the oxygen usage of the subject during the periods of rest which follow his exertion. We shall find that the rate of oxygen intake will fall off, at first relatively quickly, then more slowly, but will not reach a constant level for perhaps one and a half to two hours after the completion of the exercise. The excess of oxygen used over and above the amount corresponding to the resting requirement of the body, is spoken of as *excess oxygen*. When a curve of the excess oxygen intake is plotted against the time which has expired since the termination of the exercise (Fig. 66, p. 215), the area of the curve up to the time when the value attains a constant (*i.e.*, the resting) level will give us what may be termed the " excess oxygen usage," *i.e.*, will tell us how much oxygen has been utilised in the recovery processes of the fatigued muscle. It will be found that the total excess oxygen amounts during the whole recovery to, say, about 10 litres. The total oxidation associated with the exercise has, therefore, been 12·8 litres, of which only 2·8 litres has been used during the actual exercise. During those thirty seconds the muscles have committed the body to the removal of an amount of lactic acid which needs 12·8 litres of oxygen, but

it has only actually been able at the time to get 2·8 litres; the remaining 10 litres stands over as an "*oxygen debt*," which can be redeemed only after the work has ceased. The body has therefore, as it were, been expending its energy at a rate equivalent to a utilisation of 25·6 litres per minute, though its direct income, calculated from the oxygen used, is at most only 5·6 litres per minute.

Let us see to what amounts of lactic acid these oxygen consumptions correspond. We know that in frog's muscle when lactic acid is removed in the recovery process it is not all oxidised, but a large fraction (four-fifths) of it is reinstated as glycogen. The fraction which is oxidised is smaller, and the fraction reinstated larger, the higher the efficiency of the working muscle. It has been assumed on various grounds that in human muscle also the amount of lactic acid oxidised is, under favourable conditions, about one-fifth, while four-fifths are restored. From the equations

$$\underset{(90\text{ grams})}{C_3H_6O_3} + 3\,O_2 = \underset{(3 \times 22{\cdot}2\text{ litres})}{3\,CO_2} + 3\,H_2O$$

we see that in the oxidation of 90 grams of lactic acid with 66·6 litres of oxygen there has been a total disappearance of $5 \times 90 = 450$ grams of lactic acid, which means that with this (4 : 1) oxidative efficiency each litre of oxygen causes the removal of about 6·8 grams of lactic acid. Let us call it roughly 7 grams. Then the total lactic acid liberated during the period of exercise under consideration was $12{\cdot}8 \times 7 = 89{\cdot}6$ grams, of which only $2{\cdot}8 \times 7 = 19{\cdot}6$ grams was oxidised during the exercise, the remainder, 70 grams, accumulating in the muscles for removal during the recovery periods. If now we assume that the weight of the muscles in use in this man was, say, 25 kg., the actual concentration of lactic acid in the muscles at the end of exercise would be not less than 0·28 per cent. This is a figure which would agree pretty closely with the actually observed fatigue maximum in laboratory experiments.

We see now that the reason why an excessively violent

exercise cannot be kept up for more than about half a minute is because at the end of this time the lactic acid content has already risen to the fatigue maximum, and the muscles are consequently incapable of further effort. A little consideration will show that it can make but little difference (less than 2 litres of oxygen) to this brief but violent performance whether the breath is held at the time or not; in actual athletic performances of this short duration (*e.g.*, a 100 yards race) the breath often is held if this has, by keeping the chest rigid, any mechanical advantage.

From a consideration of their results, Hill, Long and Lupton came to the conclusion that there were two processes of lactic acid removal going on during recovery from exercise. The one is a rapid process, and corresponds with the oxidative removal of lactic acid from the muscular tissue in which it has accumulated; the other process is much slower, and is due to the oxidation of the lactic acid which has leaked away from the muscles and entered the blood stream.

The "Steady State" in Moderate Exercise.—When the exercise which is taken is of a much more moderate kind, such as walking, or running at a pace which can be kept up for an almost indefinite length of time, the conditions are very different.

Let us consider the muscle at the commencement of such a spell of activity. It contains, to begin with, practically no lactic acid, and its oxygen requirement is also at the very low resting value. When the contractions commence, lactic acid is formed at a greatly increased rate; as soon as this happens the oxygen consumption by which it is removed is likewise accelerated; as the lactic acid continues to be formed, so the oxygen intake continues to grow, for, as we have already learned from the study of isolated muscle, the velocity of the recovery process (oxygen usage) increases with the extent of the initial break-down (lactic acid accumulated). Hence the more lactic acid there is accumulated in the muscle, the faster becomes the oxidative removal, which

tends to prevent any further increase. Ultimately (assuming the exercise to be moderate) the speed of the recovery process becomes equal to that of the break-down process. This is shown by a study of the curve for oxygen intake from time to time during the exercise. The oxygen intake per minute rises from the resting level, at first quickly, then more and more slowly, finally to reach a uniform rate which can be

FIG. 67.

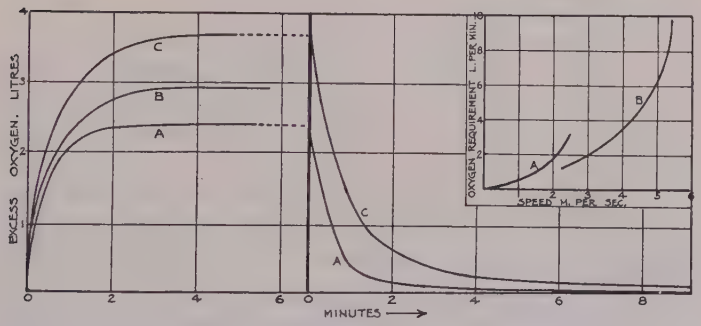

FIG. 65. FIG. 66.

FIG. 65.—Excess oxygen intake at commencement of work of different degrees of severity. In A and B the steady state is reached. In C the limit of supply is reached. (After Hill and Lupton.)

FIG. 66.—Recovery excess intake of oxygen after work. A and C represent recovery from work comparable with that in the curves A and C of Fig. 65.

FIG. 67.—Relation between speed of locomotion and oxygen requirement. Curve A, walking; curve B, running. (After Hill, Long and Lupton.)

kept up for a considerable length of time (Fig. 65, A and B).

This uniform rate is reached when the lactic acid content of the muscle has risen high enough to provoke an oxygen usage great enough to keep pace with the lactic acid production. It is called the "*steady state*," and signifies the attainment of a steady rate of oxygen usage, together with the upkeep of a constant content of lactic acid in the muscle by balance of the rates of formation and removal. The more

severe the exercise, the higher will be the oxygen usage and lactic acid content when the steady state has been reached (Fig. 65). In running long-distance races it is the practice of trained athletes to run the first few laps very quickly. The physiological meaning of this is that by so doing the steady state is reached more rapidly, so that the runner begins maximal oxygen intake and maximal recovery processes at the earliest possible moment.

Even in mild exercise leading to the establishment of a steady state there is still an oxygen debt, though it may be only a small one, for when the exercise ceases the oxygen usage does not instantly fall to the resting level, but slowly subsides as the lactic acid present in the steady state is oxidised away (Figs. 65 and 66).

Comparison of Moderate with Violent Exercise.—It will be evident from what has been said that the one form of exercise gradually merges into the other. As the severity of the exercise is increased, so the oxygen utilisation necessary to enable the steady state to be kept up will augment, and the amount of lactic acid present during the steady state will also become greater with increasing effort. But so long as the recovery process can ultimately catch up with the break-down process, *i.e.*, so long as the oxygen (blood) supply to the muscles is adequate, the lactic acid remains at the level value of the steady state, so that the total amount present and the corresponding oxygen debt are not great. Such exercise can be kept up for very long periods. But if the exercise becomes really severe, the lactic acid is liberated at such a rate that the oxygen supply reaches the limiting value and cannot be further increased; then the steady state is not possible, and lactic acid accumulates more and more, and finally brings the performance to an end as soon as the fatigue maximum has been reached. In such cases the oxygen debt is a very heavy one, and the process of recovery is prolonged (Fig. 65, curve C, and Fig. 66, curve C). Hill, Long and Lupton find that the total excess oxygen can be

estimated with sufficient accuracy if the expired air be collected during the first thirty-five minutes of recovery and the oxygen excess determined from analysis of this, with deduction of the oxygen usage during complete rest.

The rapid increase in the oxygen requirement as the severity of the exercise is increased is shown in Fig. 67, in which the oxygen requirement is plotted against speed of walking (curve A) or running (curve B) in metres per second. It is seen that, whereas in walking the requirement is not likely to surpass the means of supply, this does not hold true for running, in which at speeds over about 4 metres per second there will be a rapidly increasing oxygen debt, for the requirement can easily mount up in this subject to over 9 litres of oxygen per minute, an amount which could not be provided at the time. The uneconomical character of rapid running is no doubt in part to be explained as due to loss of energy in overcoming the viscosity of the muscle substance, as discussed on p. 177.

The following table gives a comparison of the oxygen debts associated with exercise of varying severity :—

Oxygen Debts in Exercise.

(From Hill, Long and Lupton.)

Exercise.	Duration of Recovery, minutes.	Oxygen Debt, litres.
1. Running at 3 metres per second for 5 minutes	9·5	1·7
2. Running at 2·7 metres per second for 1 hour	60	4·3
3. Standing running as violently as possible for 20 seconds	14	5·5
4. Running 225 yards in 23·4 seconds	27	8·7
5. Jumping for 2·1 minutes	24	10·5
6. Quarter-mile run, followed by severe gymnastic exercise	44	12·4
7. Standing running for 4 minutes (breathing oxygen)	87	18·6

It will be seen that in examples 1 and 2, where the exercise was of a mild character, the oxygen debt was relatively small, even although in the second case the exercise had been continued for an hour. But in example 3 very violent exercise even for a few seconds can give as large an oxygen debt as the more moderate form in one hour; examples 4 and 5 bear this out even more strongly, since the exercise was maximal; *i.e.*, the subject was quite exhausted at the end of the period. In examples 6 and 7 we see that the oxygen debt can acquire large proportions when the exercise is both violent and relatively prolonged.

Calculating from the assumption that each litre of oxygen used in recovery effects the disappearance of 7 grams of lactic acid from the tissues, the following calculations of the lactic acid concentrations in the muscles at the termination of exercise have been made by Hill, Long and Lupton :—

Oxygen Debt and Lactic Acid of Muscle.

Oxygen Debt, litres oxygen per kg. body weight.		Estimated Lactic Acid Percentage in Muscle.		Lactic Acid per kg. body weight.	
Subject breathing Air.	Subject breathing Oxygen.	Subject breathing Air.	Subject breathing Oxygen.	Subject breathing Air.	Subject breathing Oxygen.
0·115	0·150	0·265	0·345	0·70	1·05
0·103	0·168	0·237	0·387	0·74	1·17
0·216	—	0·51	—	1·5	—
—	0·266	—	0·62	—	1·86

These figures show that in a man of 70 kg. body weight the total lactic acid formed during severe exercise may be of the order of 100 grams. Even if we assume that as much as one-third of this has been able to diffuse away into the blood stream, there would still remain enough in the muscles to give a percentage of 0·3 to 0·4 per cent., a figure which agrees well with the fatigue maxima which have been found

for frog's excised muscles. In the severest forms of exercise, lactic acid is formed at the rate of about 3 grams per second, so that a maximal effort can only last for about thirty seconds.

It will be noticed from the results given in the above Tables that a higher oxygen debt can be contracted when oxygen is breathed than when air is breathed. In these experiments it was found that the oxygen intake during the exercise was also raised, as might be expected, and yet, in spite of this, a greater oxygen debt could be contracted. The explanation seems to be that when oxygen is breathed the distress is for some reason not felt so soon, so that the effort can be kept up for a longer time than when air is breathed.

The Oxygen Intake during Severe Exercise.—We have seen that after exercise has commenced the oxygen intake rapidly rises until either a steady state has been reached or until the limiting value of oxygen intake is attained, the former happening in more moderate, the latter in severe, exercise. In either instance the oxygen intake remains constant, or practically constant, so long as the exercise is continued unabated. This constancy is due, when the steady state is reached, to the oxygen intake just balancing the requirement from moment to moment ; but if the requirement exceeds the possibilities of supply, so that the steady state is not reached, the constancy is due to the fact that the conditions of supply, though inadequate to meet the demands, remain constant.

It follows that the difference between moderate and really severe exercise is that in the former an increase of the severity of the exertion will cause an increase or reduction in the contemporary oxygen intake, while with very severe exercise increase or small reduction of it will cause no appreciable change in the oxygen usage, because this is already maximal and is all that the conditions of the circulatory and respiratory systems will allow.

Inspection of the results of many investigations on the oxygen usage during exercise shows that different maximum

intakes have been recorded for different forms of exercise. In most of them the maximum of oxygen intake is much smaller than in rapid running. The following table illustrates the maxima obtained by different workers :—

Exercise.	Litres Oxygen per minute.	Observers.
Bicycling .	3·0	Benedict and Cathcart.
,, .	2·8	Campbell, Douglas and Hobson.
,, .	3·2	Lindhard.
Swimming .	2·8	Liljestrand and Stenstrom.
Skating .	3·1	,, ,,
Skiing .	3·8	,, ,,
Running .	3·5	,, ,,
,, .	4·2	Hill, Long and Lupton.

The reasons for the high oxygen intake during fast running are that (*a*) the requirement is high, because the mass of muscle in use is large ; (*b*) the conditions for intake are optimal, because the respiration and circulation are not impeded, as they must be to some extent in cycling experiments. We may assume, then, that a healthy man of, say, 70 kg. body weight, can under the most favourable conditions attain an oxygen intake during exercise of about 4 litres per minute. Any requirement in excess of this will involve the contraction of an oxygen debt the magnitude of which will be proportional to the duration of the exercise.

An oxygen intake of 4 litres per minute would necessitate a very high circulation rate. If we assume a mean coefficient of oxygen utilisation of 0·66, then, if the arterial blood contains 14 per cent. oxygen (*i.e.*, is assumed to be 90 per cent. saturated, and of 18·5 per cent. capacity), the blood flow through the lungs must be of the order of $\frac{4{,}000}{0{\cdot}66} + \frac{100}{17}$ c.c., or about 36 litres per minute. When oxygen was breathed, the circulation rate in Hill, Long and Lupton's

experiments was calculated to be in some cases over 40 litres per minute, or an output of about 200 c.c. per beat.

The Carbon Dioxide Output and the Respiratory Quotient

The Carbon Dioxide Output during Exercise.—As soon as the exercise is commenced the ventilation of the lungs is increased, and this deepened breathing continues throughout the exertion, and is prolonged well into the recovery period. The immediate increase in the depth of breathing is due, as Krogh and Lindhard have shown, to psychical causes associated with the effort, but the continued large intake of air is to be explained on the ground of a persistent chemical stimulus to the respiratory centre. Whether this stimulus is to be found in a rise in the hydrogen ion concentration of the blood or whether carbon dioxide, acting as a specific stimulus to the centre, produces the excitation, or whether in extreme exertion oxygen lack may also figure in the process, we cannot discuss here. The experiments of Campbell, Douglas and Hobson made it clear that, coincident with the increased ventilation, there was in all probability an increase both in the carbon dioxide pressure and inferentially in the hydrogen ion concentration of the circulating blood. They also found, as others had done before, that the respiratory quotient rose during the exercise, showed a further sharp rise on its conclusion, and then fell to a low value during the subsequent recovery period.

The pronounced rise of respiratory quotient during exercise cannot be entirely explained on the ground of a contemporary oxidation of lactic acid, for such an oxidation would give a respiratory quotient of 1, whereas the respiratory quotient during the exercise is often greater than unity.

The highest values of the respiratory quotient both during and after the exercise are seen in running, and we have seen that in these experiments the oxygen debt was also highest.

Furusawa finds that in running the respiratory quotient attained after the first few minutes increases regularly with the speed of movement.

The rapid rate of output of carbon dioxide which gives rise to these high respiratory quotients must be attributed

Respiratory Quotients before, during, and after Exercise.
((*a*), (*b*), (*c*), etc., represent successive periods.)

Respiratory Quotient before.	Respiratory Quotient during.	Respiratory Quotient after.	Observers.
0·84	0·87	0·75	Benedict and Cathcart (cycling)
0·85	0·90	0·78	,, ,, ,,
0·79	0·82	0·75	,, ,, ,,
0·83	1·002	(*a*) 1·17 (*b*) 0·89 (*c*) 0·76	Campbell, Douglas and Hobson (cycling)
—	1·03	(*a*) 1·132 (*b*) 1·255 (*c*) 0·651	Campbell, Douglas and Hobson (cycling)
—	(*a*) 1·34 (*b*) 1·63 (*c*) 1·87	(*a*) 2·08 (*b*) 1·73 (*c*) 1·58 (*d*) 1·22 (*e*) 0·93 (*f*) 0·85	Hill, Long and Lupton (running)

in part to the greatly increased ventilation rate. Thus high quotients can be obtained by forced breathing while the body is at rest, and are due to the greatly accelerated rate at which carbon dioxide is washed out from the blood and tissues.

During the exercise, however, another factor is at work, viz., the rapidly accumulating lactic acid. The experiments of Hill, Long and Lupton have shown that in severe exercise

lactic acid accumulates in relatively large amount in the blood plasma, and this must lead to an accelerated output of carbon dioxide. We must remember that during the most intense muscular effort lactic acid is being set free at the rate of about 3 grams per second, and if for the moment we regard this amount as displacing an equivalent amount of carbon dioxide, there would be an expulsion of 0·73 litre of carbon dioxide per second (*i.e.*, about 0·245 litre per gram) in addition to that set free by contemporary oxidations.

Actually, it would probably be incorrect to assume that the carbon dioxide is directly displaced from its combination as bicarbonate. What actually happens is apparently that the acid as soon as it is formed is neutralised by the sodium protein compounds. The result of this is, according to Hill, Long and Lupton, to raise the hydrogen ion concentration of the blood, so leading to increased ventilation, which washes out the equivalent of CO_2. The net result is much the same.

The lactic acid production is, however, so rapid that the lungs are unable, in spite of the increased ventilation, to wash out the carbon dioxide fast enough, so that there is an accumulation of it in the body during severe exercise. Thus in twenty seconds a man can produce, say, 60 grams of lactic acid, of which he can perhaps oxidise away some 10 grams at most. The remaining 50 grams would displace about 12 litres of carbon dioxide, but such a large amount could not possibly be eliminated from the body in twenty seconds, for this would imply a content of 30 or more per cent. of CO_2 in the expired air.

The Carbon Dioxide Output After Exercise.—We can now understand the sharp rise in the respiratory quotient which is so often seen to follow the conclusion of severe exercise. When the exertion ceases, the oxygen intake is very rapidly reduced, as seen from Fig. 66, but the elimination of the carbon dioxide must still continue until the H-ion concentra-

tion of the blood has been reduced to the resting level. For this reason, the respiratory quotient at first shows a brisk rise. When the blowing-off of CO_2 has been sufficient to restore the H-ion concentration of the blood to a normal value, the washing-out process stops, and respiration falls practically to the normal.

When this stage has been reached, there still remains a considerable amount of lactate in the blood and tissues, and as this undergoes oxidation in the course of the recovery process, liberating the base again, it follows that the reverse of the above process must set in, as otherwise the tissues would be left finally in too alkaline a condition. If we postulate that for each gram of lactic acid there has been, during and immediately after the exercise, an expulsion of about 0·245 litre of CO_2, then, as soon as this liberated CO_2 has been removed from the body, we must suppose that each gram of lactic acid removed will require the retention of a similar 0·245 litre of CO_2. We have seen that 1 litre of oxygen causes the removal of about 7 grams of lactic acid, of which about one-fifth (1·4 grams) is actually oxidised, yielding 1 litre of CO_2 :—

$$C_3H_6O_3 + 3\ O_2 = 3\ CO_2 + 3\ H_2O.$$

In these later stages, therefore, the 7 grams of lactic acid so removed would necessitate a retention of $7 \times 0{\cdot}245 = 1{\cdot}7$ litres of CO_2. Hence there would be 1 litre of CO_2 produced by oxidation, while there would be retention of 1·7 litres, the net retention being thus 0·7 litre. In other words, there is retention of 0·7 litre of carbon dioxide formed elsewhere in the body to make up the loss of carbon dioxide which has been effected by the production of lactic acid, and expulsion in the earlier stages of exercise and recovery. The minimal value of the respiratory quotient due to this retention occurs after severe exercise, at the end of about seventy or eighty minutes of recovery.

The Efficiency of Recovery.—We have just calculated that

during the later stages of the recovery process there is a retention of about 1·7 litres of CO_2 for each litre of oxygen used in the recovery process; *i.e.*, for each litre of excess oxygen there is a net deficiency of· 0·7 litre of the CO_2. In other words, the CO_2 output of the body is less than the resting value by that amount. This is on the assumption that in the recovery process four-fifths of the lactic acid is replaced to glycogen, while one-fifth of it is destroyed by oxidation. Hill and his co-workers have found that in the later phases of the recovery process there is actually a similar ratio between the excess oxygen and the excess carbon dioxide, corresponding with the fall in the respiratory quotient to a value lower than that in rest. The efficiency of recovery is not always exactly of this order, but may be calculated in any particular case from the formula

$$\text{recovery efficiency} = \frac{3 \times (\text{volume of carbon dioxide retained})}{(\text{volume of recovery oxygen})}$$

in a given time.

A retention of 1·7 litres of CO_2 per litre of recovery oxygen gives $3 \times 1{\cdot}7 =$ about 5; *i.e.*, one-fifth of the lactic acid is oxidised, and four-fifths restored, thus agreeing with the findings for isolated frog's muscle under favourable conditions.

The "Mechanical Efficiency" of Human Exertion.—It is possible to calculate the amount of mechanical energy liberated (theoretical maximal work) by men carrying out various exercises under normal conditions, and then by determining the oxygen utilisation during the work and recovery, to compute the mechanical efficiency of the performance. Many approximations have to be made and sources of error allowed for, but it is surprising with what constancy an experienced athlete can perform. In running, which has been chiefly studied, the allowances to be made include those for starting up and stopping and for onset of fatigue. In running, little actual mechanical work is done,

the energy set free being used for imparting velocity to the body, a good deal of it being lost in overcoming viscous or other resistance in the muscles and other structures. The speed reached depends on the ratio of propelling force to resistance, chiefly that of his own muscles, as viscosity, and that represented by starting and stopping the movements. The resistance of the air is normally small, and may be allowed for. In still air it is about 5 per cent. of the total, and at a velocity of 30 feet per second is about equal to 5 lbs. When a known resistance is put against the runner, the effect in reducing the speed ultimately reached is identical with that accounted for as internal resistance in the muscles, etc. (Best and Partridge). Similarly, for bicycle pedalling, Mrs. Dickinson has shown that the maximum speed reached varies in linear relation inversely with the load. The force F, necessary to impart this speed to the body in running against this resistance is $= f\,\mathrm{M}\,g$, where M is the mass of the runner, f a constant, depending on subject, and representing the value of the force in terms of the body-weight of the subject. Usually f lies between 0·5 and 1·0. The work done in running a distance l, $= f\mathrm{M}gl$. The value of f, and hence the propelling force, can be determined by an equation of motion, from direct observation of the times at which different distances are covered. In an examination of some 1928 Olympic champion male and female sprinters, Best and Partridge found that f was greater for men than for women, *i.e.*, men's muscles form a larger fraction of their body weight. The superiority of first-class athletes over others of equal muscular strength appears to be due to the smaller " viscosity " of their muscular apparatus, using this term in its widest sense to mean that in them less force is wasted in overcoming the resistances represented by putting their limbs in motion. This conclusion is perhaps implicit in the statement that one individual is a faster runner than another, but it is interesting to note that it is not a matter of muscular power alone. The viscosity constants for women appear to

be much the same as in men. The speed attained depends on both f and the viscosity coefficient. Suppose that f thus determined represents 0·7 of the body-weight, the body-weight = 161 lbs., and the distance to be run is 60 yards, plus 5 yards allowance for pulling up, then the calculated work is $161 \times 0{\cdot}7 \times 65 \times 3 = 22{,}000$ foot-lbs. (If the distance is covered in 7·6 seconds, this = 2,890 foot-lbs. per second, and since 1 h.p. = 550 foot-lbs. per second, the power = 5·26 h.p.) When the work done is thus calculated and the efficiency determined by finding the fraction of the oxygen utilisation which it represents, it was found by Hill that in running distances from 60 to 150 yards the efficiency was of the order of 38 per cent. If we allow for loss due to the contraction of muscles not engaged chiefly, or only inefficiently used, in propulsion of the body, it would follow that the efficiency of the muscles which are used for propulsion must be well above 40 per cent., *i.e.*, not far from the total energy liberation which corresponds to the initial processes of the contraction.

Now we have already learnt in the last chapter that when the *realisable* work is taken into account, whether the movements be rapid or slow, the maximal efficiency is never greater than 25 per cent., and even this level of efficiency is only reached under optimal conditions, for the reasons, already emphasised, that much of the energy is used either in overcoming viscous resistance in the muscles when the movements are quick, or in maintaining the contractions when these are prolonged. In the calculations just made, these factors are eliminated.

Nature of the Material consumed in Contraction.—Nearly all who have worked on the respiratory exchanges in connection with exercise have speculated as to the nature of the substance or substances which supply the muscle with its energy. The fact that the respiratory quotient frequently rises to about unity in exercise experiments has led many to believe that carbohydrate is the source of muscular power.

But if we are to accept this criterion without question, we would have to infer that the quotients higher than unity during exercise implied a change of some other description.

We have seen that an explanation of the variations of the respiratory quotient during exercise can be supplied by the application of elementary facts learned from experiments on the nature of the chemistry of muscular contraction. Above all, we have seen that the proper measure of the energy expenditure associated with a piece of hard muscular work can be best derived not from the study of the contemporary oxygen usage, but by finding the total oxygen requirement, as seen in the combined exercise and recovery.

Similarly it is reasonable to suppose, particularly when we bear in mind the fluctuations of the respiratory quotient which have been explained, that the clearest light on the total metabolism of contraction would be obtained by the study of the total excess oxygen and excess CO_2 during the entire exercise *plus* recovery.

Respiratory Quotients of Exercise and Recovery

(Hill, Long and Lupton).

"Excess" during Exercise *plus* Recovery.		
Excess Oxygen Litres.	Excess CO_2. Litres.	Respiratory Quotient.
2,130	2,210	1·04
2,320	2,280	0·98
2,849	2,655	0·93
4,117	4,580	1·10
5,200	5,450	1·05
	Mean R.Q. =	1·02

When this is done it at once becomes clear (see Table above), that the excess metabolism has a respiratory quotient of about unity. This probably means, in the long run, that carbohydrate is the source of the energy of contraction in human, as it has been shown to be in frog's, muscles.

The question arises whether fat can also be utilised to supply energy for muscular contractions. Krogh and Lindhard found that subjects fed with a fat diet were much more readily fatigued than those fed on carbohydrate. When a subject passed from the state of rest to moderate muscular work, it was found that the respiratory quotient was raised if it had been previously low, but lowered if it had been previously high. They interpret their results by suggesting that the proportion of fat to carbohydrate used, whether at rest or during work, depends on the available supplies of both; when the diet consists chiefly of fat (respiratory quotient below 0·8), there is a constant conversion of fat into carbohydrate, which is provisionally stored, while when the respiratory quotient is above 0·9 carbohydrate is being transformed into and stored as fat. These conversions involve an expenditure of energy in consequence of which the basal metabolism is a few points per cent. higher when the respiratory quotient is abnormally high or abnormally low than when it is normal. The respiratory quotient can be considered as the resultant of two processes: a catabolic (combustive) and an anabolic (transformative). Transformation of fat into carbohydrate involves retention of oxygen, and this lowers the catabolic respiratory quotient still further; conversely, conversion of carbohydrate into fat, by making oxygen available, makes the basal respiratory quotient still higher. During work the catabolic processes are increased more than the anabolic ones, and hence the low respiratory quotient of the fat-fed subjects is raised, because the carbohydrate formed from the fat is now being burned faster than it is generated. Similarly, when the resting respiratory quotient is high, it falls during work, because now carbohydrate is rapidly burned, and less is being converted into fat. It seems plausible, then, that if the muscle does consume fat, it does so by first converting it into carbohydrate, in which process there is some loss of energy.

Experiments by Furusawa have shown that in individuals on normal diets during short periods of muscular exercise the respiratory quotient of the excess metabolism is unity. When a fat diet was used it was found that even when the basal respiratory quotient was 0·71, short periods of exercise gave a respiratory quotient of unity for the excess metabolism, but when the exercise was more prolonged, the fall took place sooner than on normal diets. It would seem from these experiments that carbohydrate is the real fuel which is used by the contracting muscles, and that when the carbohydrate store has been all used up other substances, such as fat, can be called upon, probably being converted into carbohydrate.

It might be expected that the rapid utilisation of carbohydrate reserves would result in lowering of the blood sugar when exercise has been very prolonged. This result is opposed, however, by various processes which accelerate the mobilisation of such actual glycogen reserves as the body has, and further by the increased gluconeogenesis just referred to. In extreme and prolonged effort, the rate of new formation may fail to keep pace with the rapid usage of sugar, and definite hypoglycæmia may be present. This is the explanation given by Best and Partridge, who found that in three out of ten runners in the " marathon " race (about 26⅓ miles) in 1928, the blood sugar level had fallen below the normal. In shorter efforts the blood sugar may quite well be above the normal owing to over-mobilisation.

Hetzel and Long have shown that exercise in the diabetic subject is accompanied by essentially similar phenomena, which indicates that even under these conditions carbohydrate is utilised by the contracting muscles. Because the store of carbohydrate is small in the diabetic, the respiratory quotient of the excess metabolism soon falls below unity if the exercise is prolonged.

It has more recently been discovered that respiratory quotients of unity for the excess metabolism are only

obtained when the exercise is of moderate severity; with very mild exercise the quotient is lower, and with extremely severe effort higher, than unity, reaching in some cases a value even as high as 1·7. It is difficult to understand what the significance of these results might be. Best, Furusawa and Ridout are inclined to reject the suggestion that the abnormally high quotients in severe exercise are due to expulsion of preformed CO_2. If that is so, it does not seem possible to bring these high respiratory quotients into accord with known facts of metabolism. A conversion of carbohydrate into fat, which would be the orthodox explanation, would appear to be extremely unlikely. The transition from respiratory quotients of about the basal level for mild exercise to the value of unity for moderate exercise is perhaps understandable enough when we bear in mind that the respiratory quotient must be regarded as the algebraic sum of all the processes which lead to the intake of oxygen and expulsion of CO_2 and would indicate progressively increasing utilisation of carbohydrate up to that point.

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CHAPTER IX

THE NERVE IMPULSE

Every nerve fibre under normal conditions in the body can conduct along its axis cylinder a propagated disturbance known as the nervous impulse. Although under normal circumstances the nerve impulses along each fibre are generated in a specific place, the cyton, or the sensory terminal, yet we know that a nerve is capable of excitation by artificial means at any point in its course. The result of such a stimulus appears to be the liberation in many of the fibres of volleys of nerve impulses, starting from the point of excitation and running with equal velocity in both directions. These nerve impulses are characterised and accompanied by, and perhaps actually due to, electrical changes propagated along the axis cylinder, and lasting at any given point for a very short time, normally about 0·6 to 0·8σ or even less.

When by prolonged stimulation the nerve is thrown into protracted activity, a study of these action potentials indicates that the excitatory changes in the nerve fibre are interrupted, not continuous, even though the stimulus which gives rise to them may be quite continuous. We may compare the passage of such a stream of nerve impulses to the discharge of a stream of machine-gun bullets, rather than to the flow of a stream of water; "continuous" activity of the fibre being represented by a quick succession of these short waves. The explanation of this rhythmic discharge is that excitation is followed by a refractory period and a second response cannot be obtained until this has subsided.

Afferent and Efferent Impulses.—Investigations have shown that there is a uniform type of response in all kinds of nerve fibres, whether these are afferent or efferent; in all cases the response consists of one or more nerve impulses. This is so in the natural discharge of impulses along the motor nerve; such impulses can be brought about voluntarily or reflexly, as well as by direct stimulation of the nerve trunk, and there is no fundamental difference between the naturally and artificially induced impulses.

It is not so easy to investigate natural stimulation of sensory nerves, because it is more difficult to stimulate a

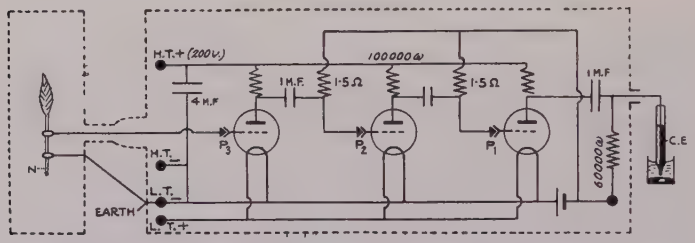

FIG. 68.—Three-valve amplifier employed by Adrian. The capillary electrometer CE is led off from the third valve. The nerve is laid on the leads as shown. P_1, P_2, P_3 = plug-in keys.

sufficiently large number of sensory fibres simultaneously in the normal manner, *i.e.*, by the application of stimuli to the nerve-endings. The electrical potential difference set up by the action currents in a nerve trunk depends upon the ratio of active to inactive fibres, and hence is immeasurably small for ordinary methods of observation when normal sensory nerve impulses are generated and the sensory or mixed nerve led off to a detecting instrument. Einthoven and Buytendijk carried out some experiments in this direction, and Alexander Forbes was able by the use of valve amplifiers to demonstrate action currents in muscular nerves when the muscle was stretched; these action currents presumably represented nerve impulses set up in the muscle

proprioceptors. The use of valve amplification with detection of the magnified potential differences by the capillary electrometer, has been used with good results by Adrian and his pupils.

Using an amplification of about 1800, Adrian was able to show that when the frog's gastrocnemius muscle was stretched, showers of impulses passed up the sciatic nerve. Similarly it was shown that there is a continual stream of afferent impulses from the heart, passing along the depressor and vagus nerves, some of the impulses along the latter coming also from the lungs. Adrian and Zotterman, with the same arrangement, were able to show streams of impulses passing up the sensory nerves, from the pad of the cat's foot when this was subjected to pressure or other cutaneous stimulation, and Adrian and Matthews showed similar effects in the optic nerve of the eel when the eye was exposed to light. These researches indicate that the normal impulses travelling along sensory nerves are of the same nature as those which normally travel down the motor nerves.

Analysis of Afferent Impulses.—The afferent impulses aroused by stimulation of many nerve endings, as for example those transmitted along the sciatic nerve when the gastrocnemius is stretched, represent simultaneous impulses along many nerve fibres. It is desirable further to analyse such results, and so to study the impulses passing along a single nerve fibre, from a single terminal. Adrian and Zotterman investigated the afferent impressions from the sternocutaneous muscle of the frog, the nerve supplying which has about 18 fibres. When the muscle is pulled, *e.g.*, by a weight of 2 grams, the resulting electric response suggests superposition of impulses in several fibres. In order to reduce the number of fibres, strips of the muscle were cut away, and the remainder stretched, until eventually a fragment remained, the stretching of which generated a single succession of quite regular impulses at a frequency of from 5 to 100 per second, the intervals between impulses depending

upon the *intensity* of the stimulation to which they were due—thus, when pulled with a weight of one gram, the frequency of impulses (from a piece of muscle containing at least two end-organs) was about 290 per second ; with half a gram 200 per second ; with one quarter of a gram about 150 per second, each individual impulse being of the same size.

By similar methods, Adrian and Umrath have examined the different impressions produced by stimulation of the Pacinian corpuscles found in relation to the flexor tendons of the cat's toes ; these they find to be stimulated by pressure, but not by temperature changes. The rate of emission

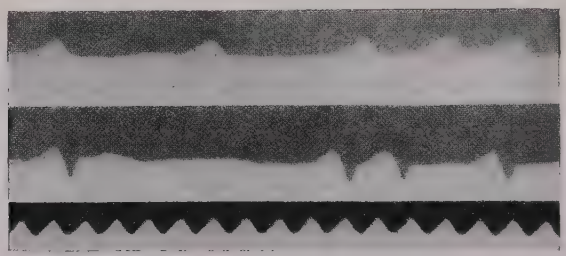

FIG. 69.—Oscillograph. Records of action potentials in nerve by stimulation of sensory endings in toe muscle of frog by stretching by a weight. A = nerve injured (monophasic). B = nerve intact (diphasic). C = time trace 200 d.v.p. sec. (Matthews).

of impulses varies from 5 to 100 per second, according to the intensity of the stimulus.

We have thus, in response to stimulation an " all-or-none " response, but at a rhythm becoming faster as the strength of stimulation is augmented. This was seen very clearly when the loading of the muscle was gradual, the result being a steadily increasing frequency of discharge.

Characteristics of Response from Nerve Endings.—When a prolonged constant stimulus is applied to a nerve trunk there is an impulse at the inauguration of the excitation and one at its termination, with a quiescent condition in between. In contrast with this, continuous stimulation of the nerve-

ending generates a train of impulses along the nerve trunk, and in some cases this may be maintained during a long period of stimulation. The question arises why a continuous train of impulses is generated by the nerve-ending; better expressed perhaps in another form, why does the nerve not generate such a train of impulses? The answer probably is that in the nerve, owing to processes of adaptation, the excitatory effect speedily dies away; if, however, a *strong* stimulus is given to a nerve it is possible to generate a short and rapidly dwindling train of impulses. Adaptation to the stimulus is evident also with some types of nerve endings, the frequency of the discharge sometimes falling off rapidly as the stimulus is continued—the afferent excitations arising from stretched frog muscle, for example, only fall to 70 per cent. of their initial frequency after 3 seconds, whereas that from hair terminals in response to bending of the hair, dies away completely in one second, and that from pressure on the pad of the cat's foot dies away in one-fifth to one-tenth of a second. This falling-off in frequency is due to adaptation and not to fatigue processes, because we find, for example, that whereas sudden pressure gives big responses, gradual pressure gives smaller responses, but ultimately they both die down to the same level.

It appears from investigation of the frequency of response, that the refractory period of the nerve fibre does not enter into the physiology of sensory reception, since the end organ emits impulses more slowly than the fibre, and hence the fibre is always able to carry all the impulses which the end-organ can generate. Apart from this difference, the physiology of the end-organ and of the fibre appears to be essentially the same.

Impulses along the optic nerve have been studied by Adrian and Matthews in the conger eel, which has a long and simple optic nerve. When light falls on the eye there is a latent period of about 0·2 second, followed by a burst of impulses. If the intensity of the light is increased, the heap of impulses

mounts higher. When the light is turned off there is a renewed outburst of impulses. The fundamental question is how light acts on the receptive terminals of the retina. The length of latent period suggests that there is some intermediate phenomenon, and this is supported by facts derived from the study of electrical changes of the retina by Einthoven and Jolly, for a discussion of which Adrian and Matthews' paper should be consulted.

The speed of adaptation in various end-organs is correlated with their functions. For example, muscle spindles, pressure sense and fine touch for hairs show increasing speeds of adaptation, and the reflexes normally elicited from these terminals show corresponding properties. The postural reflexes elicited from the muscle spindles are tonic, and do not exhibit fatigue. Phasic reflexes, on the other hand, show fatigue, and are elicited from quickly adapting structures, such as the hairs. In general terms, the more nearly an end-organ approaches in structure to a naked nerve fibre the more rapid is its process of adaptation. Discrimination in such naked fibres or end-organs is slight, because the excitation produced so quickly dies down. This is what happens in the simplest end-organs, *e.g.*, those of pain : the only result of an alteration in the strength of the stimulus is a difference in the duration of the resulting shower of impulses, but with muscle spindles, discrimination is finer, the whole shower lasting longer, and stronger stimulation giving more frequent impulses.

Relation of Impulses to Resulting Sensation.—There is no difference in the character of the impulses set up by different kinds of stimuli, nor in their range of frequency, which varies from about 5 per second to 100 per second in all varieties. The chief difference for different kinds of sensation would appear to be the duration of the train of impulses which is generated—for example a light prick applied to the frog's skin gives a shower of impulses, lasting for one-tenth of a second, whereas a heavier prick gives a much longer discharge.

In the skin of the frog the only receptors we know are terminal naked ramifications of nerve fibres, so that there can be no question of the different sensations (touch and pain) being generated in different terminal structures. Pressure has, no doubt, separate endings in the skin, and the process has different time relations.

The qualitative differences in sensation which result from the arrival at the central nervous system of trains of impulses of varying length, might be due to the fact that longer trains could penetrate, by facilitation, to portions of the central nervous system, which would not be reached by shorter trains. This probably applies to the quantitative difference between protopathic and epicritic sensations.

The relation of nerve processes to sensation is largely a psychological problem, but from these experiments it seems that the intensity of a sensation is determined by the number of impulses arising in a given time. For instance, the curves of intensity of sensations resulting from varying brightnesses of light very closely resemble those from the eel's eye when the frequency of the nerve impulses is plotted for different degrees of illumination, both sets of curves dying off in a similar way by adaptation. Similarly the sensation resulting from pushing a needle slowly into the skin shows a rise and falling off, which quantitatively resembles the curve of impulse frequencies in the cat, when the pad of the foot is similarly treated. Sensations, of course, do not exhibit the discontinuity shown by the nerve impulses, since by some process, of the nature of which we are entirely ignorant, the responses become fused in the sensorium to produce a continuous sensation.

Motor Impulses.—The inquiry into the nature of the impulses normally transmitted by motor nerves has in the past met with serious obstacles. A more powerful contraction of a muscle might be the result of the arrival of more frequent nerve impulses along all the fibres, or of impulses at the same rate but of greater magnitude, or of the implica-

tion of more nerve fibres, or be the result of grouping of impulses into synchronous or nearly synchronous volleys at a particular rate in fibres in which impulses were previously out of phase.

Sherrington and his pupils have shown that the examination of simultaneous records of the mechanical and electrical responses of the muscles involved in reflex movements, and the comparison of these with the results of direct stimulation of the motor nerve, provide valuable data regarding the nature of the excitation provided by the nerve centres, and operating through the motor nerve fibres upon the muscle.

Nerve impulses travelling simultaneously along numerous fibres of a nerve trunk are known as a volley. The term "synchronous volley" is applied to a burst of impulses travelling in a group so that the impulses passing along different fibres arrive at points the same distance from the centres at the same time, and hence activate the muscle fibres at approximately the same time. A volley of impulses arriving at the muscle will produce a single twitch, *i.e.*, a contraction of a particular brief duration, *e.g.*, about 40 σ for certain muscles of the cat. An "asynchronous volley," *i.e.*, a group of impulses arriving at their goals at slightly different times, would give a twitch of about the same duration, or very little greater, but of slightly different form, and exhibiting a more prolonged electrical response of the muscle, owing to the slightly asynchronous response of its individual fibres.

When the nerve trunk conveys a series of two or more volleys, the resulting contraction is a tetanic one, as shown by its greater duration. Thus for two volleys close together the mechanical response is about half as long again as for a single twitch, say, for the muscles mentioned above, about 60 σ. From the duration of the mechanical response, the number of volleys of impulses causing the contraction can be deduced. The myoelectric responses also give an indication of the number of volleys and of their synchronism or

asynchronism, and it is also possible to infer the intervals at which the volleys follow one another by examination of the mechanical and electrical records.

The investigation by these means of such elementary reflexes as, for instance, the knee jerk, showed that it was produced by slightly asynchronous volleys of impulses. In the spinal preparation, the knee jerk appears to be the result of four to eight volleys of impulses at the rate of about 200 per second, whereas the decerebrate preparation gives a knee jerk which more closely resembles a single twitch.

The examination of electrograms, together with mechanical records of contraction are, however, very difficult of interpretation, and as the technique available has been improved it has become increasingly clear that the complexity of the electrical changes in large masses of contracting muscle, and the high frequencies of response often inferred from them are the results of the summation of the much simpler responses at more regular and slower rates, of their constituent fibres.

One of the most instructive examples, which has been well investigated, is that of the phrenic nerve and diaphragm. This is convenient because the activity is rhythmic and the muscular response easily variable in intensity by various means, *e.g.*, by occluding the trachea, or adding CO_2 to the inspired air. Gasser and Newcomer, using valve amplification, and string galvanometer records of electro-neurogram and electromyogram, found, in the dog, that at each inspiration there was a series of responses in both nerve and muscle, at about 70 per second, the two phrenics showing action currents simultaneously. The inference was that, as had been previously suggested by Cooper and Adrian, under certain circumstances the motor neurones discharge volleys of impulses at regular intervals, with relative pauses in between. Slight irregularities in such records can be interpreted as due to a few neurones out of phase with the majority, and emitting sporadic impulses.

It would, however, be hazardous to infer from such results that the rhythm of the electric response in nerve trunk or muscle mass meant that each neurone was discharging impulses along its axon at that rhythm. More perfect experiments which support the view of synchronous action in the phrenics have been recently performed by Gasser, using the cathode ray oscillograph. He found that the individual volleys had a duration from three to seven times as great as that of an axon action potential generated synchronously in all fibres by electrical stimulation of the phrenic. The volleys, though forming quite clear groups with complete pauses between them, were therefore not quite synchronous, either because the impulses in different fibres were slightly out of phase with one another, or else because the discharge in each fibre at each volley was repetitive, *e.g.*, double or treble. The areas of the curves of the longest volleys were found, however, to be never larger than would correspond to a single response per volley in each fibre, and, usually, since not all the fibres were in activity, considerably less. Hence it was concluded that the response is a single one per axon per volley.

Still clearer evidence regarding the action currents in the phrenics was brought forward almost simultaneously by Adrian and Bronk, who applied to this nerve the technique which had already yielded to Adrian such striking results in the investigation of sensory impulses, and which in this instance enabled them to achieve what had been so long desired, the record of action currents in a single motor fibre.

Adrian and Bronk divided one by one the fasciculi of the highest root of the phrenic, which contains, in the rabbit, about 150 fibres. The nerve was cut distally and under a dissecting microscope the bundles of nerve fibres were all divided with the exception of a few fibres (1 to 4) in the middle of the nerve. The distal point of the nerve was led off to the amplifier, from which the magnified action currents

were taken to a capillary electrometer, or, for continuous observation during the dissection or experiment, to a loud speaker. Their results were striking. During normal quiet inspirations they found, when the whole nerve root was led off, that there was a rapid and irregular series of discharges, together with an indication of larger waves at about twenty to thirty a second. During forcible respiration there were large waves, fairly regular in sequence, and following one another at a rate up to 90 per second. After division of the root down to one remaining active fibre, there was seen in quiet breathing, a regular succession of impulses at the rate of about twenty to thirty per second. The phrenic, when intact, merely showed the jumbled resultant of these regular impulses, with a suggested rhythm of twenty to thirty per second, which showed that there was a slight tendency to volleying.

Increase of the respiratory movement by occlusion of the air-way caused an increase in the frequency of the impulses in each fibre, up to fifty to eighty per second, or even higher, but caused no change in the magnitude of each impulse.

With regard to the much-debated question of volleying also, their results are of considerable interest, for they find that the tendency to synchronisation in the neurons involved becomes conspicuously greater during forced than during quiet breathing. It would appear from these results that grading of contraction in the diaphragm depends mainly on alterations in the frequency of the impulses arriving along each fibre in the phrenic. When the breathing is gentle, we may suppose that each contracting muscle fibre will give an incomplete tetanus. If there were synchronisation at these low rates in all the fibres, we should get a clonic contraction, as Denny-Brown has indicated, but since the fibres do not contract together, the resultant contraction is a smooth one. When the inspirations are powerful, it may be that some new nerve fibres are also brought into play, but the chief cause of this result is the increase in frequency of impulses

in the same fibres as were previously active. At this greater rate of excitation, even though there is distinct volleying, the clonic nature of the contraction is less likely to obtrude itself, and the obvious advantage of the volleying is that it yields a more powerful contraction.

By the application of very similar methods, Adrian and Bronk have investigated the motor impulses involved in some reflex actions, the spinal flexor reflex and the decerebrate crossed extensor reflex, in response to the mechanical stimulation of pinching the foot. The flexor reflex they find is accompanied by a series of impulses, which increase in frequency from five up to twenty-five per second as the contraction develops, and the grading of the response seems to be effected by this alteration in the frequency of the impulses, and not by the alteration in the number of neurones set in action.

During the extensor reflex, and also during the stretch reflex of the quadriceps, not only does the impulse frequency increase, but there is evidence that new neurones are progressively brought into action as the reflex proceeds (recruitment). These results show remarkable agreement with those reached by entirely different methods by Sherrington and his co-workers, a summary of which has been given by Fulton.

All-or-None Character of the Impulse

We must regard the nervous impulse as some form of energy transmission, since it is capable on arrival at its destination of throwing its effector organ into activity. A very important question is that concerning the type of propagation to which the nervous impulse belongs. Is it transmitted passively along the conducting medium like a current of electricity or like sound, or does the nerve fibre undergo changes which keep the impulse alive and transmit it from point to point, as, for example, in the travelling of combustion along a train of gunpowder ?

In the former case the impulse must get progressively weaker as it travels along, and its damping down may, under certain conditions, be greatly accelerated, as, for example, that of sound in encountering a layer of cotton wool ; in other words, the nervous impulse would normally suffer a decrement which might or might not be measurable, but from which in its further passage along its course, it would never recover again.

In the latter case, the impulse would maintain a constant (all-or-none) intensity so long as the conditions were constant and, although its progress might be damped down locally, as in a stretch of burning fuse which had been moistened, it would, if and when it succeeded in emerging from such a region of depression, again flare up to its normal level.

Adrian in his investigations came to the conclusion that, after having passed through a region of impaired conduction brought about by local narcosis, the nerve impulse did again spring up to its normal level on emerging into a normal stretch of nerve ; subsequent work has thoroughly substantiated this conclusion, although it has at the same time thrown doubt on the legitimacy of some of the subsidiary arguments which led up to it. That the nerve fibre shows all-or-none phenomena is also demonstrated by an experiment by Keith Lucas ; he studied a single nerve fibre which supplied some twenty muscle fibres by branches of its axon, and found that if a stimulus was applied to the nerve fibre it either caused no contraction of any of the muscle fibres or contraction of all twenty, no grading being possible.

Adrian pointed out in 1912, that the all-or-none response of nerve is a logical consequence of its having a threshold of excitability and a refractory period. A stimulus in order to excite must produce a certain minimal local change, and the propagated disturbance which we believe is set up as soon as this change has reached its critical value, must therefore be of an all-or-none character. A stimulus beyond threshold strength " may cause the local change to exceed the value

required for excitation, but the disturbance will have been set up at the moment when the critical value was reached, and the refractory state accompanying the disturbance will prevent any subsequent change in the local conditions from affecting its size in any way."

All experimental evidence shows that the greater effect produced by a stronger, as compared with a weaker, stimulus applied to a nerve trunk, is usually due to its spread to a larger number of nerve fibres, some of which are either less excitable, or by reason of their position less accessible to the stimulating agency than others, or else, more rarely, to the production of repetitive impulses instead of a single one.

Transmission without Decrement.—Much of the work on the nature of the nerve impulse has naturally turned on the question whether the nerve normally conducts with a decrement or not. Or alternatively, can a decrement be produced or intensified artificially in a length of nerve, so as to enable us to see whether the impulse on emerging from it can recover or not ?

Observations on the effect of local anaesthesia or local cooling or pressure, were until recently thought to indicate that these agencies caused conduction with a decrement in the affected portions of nerve. The chief evidence for this belief was that in order to extinguish conduction, the agency had to be applied for a length of time, which varied inversely with the length of nerve upon which it acted. Hence it was argued, the impulse, on passing from normal nerve into a stretch of nerve brought to a particular uniform and moderate degree of, say, narcosis, must suffer a progressive weakening as it travels and ultimately, if the narcotised stretch be long enough, be extinguished, whereas if the narcotised stretch had been short enough it would still have got through. More advanced narcosis of the shorter stretch would be necessary in order to cause a steep enough decrement to extinguish the impulse in its short transit.

This view of the effect of local narcosis has now almost

certainly been disproved and replaced by the opinion that in such a region the nerve impulse is depressed as regards velocity and magnitude, but is of uniformly lowered intensity throughout, and on emergence from the region of depression rises steeply to its normal level of velocity and intensity again.

Adrian and others have shown that when a nerve is slowly narcotised and stimulated at intervals centrally to the narcotised piece, the strength of stimulus necessary to cause maximal contraction of the muscle remains constant until conduction fails, in spite of the fact that conduction is depressed in the narcotised area. This is an indication of the all-or-none phenomenon which does not depend on any assumption as to whether the conduction through the narcotised area is decremental or otherwise, but merely necessitates the assumption that conduction is depressed.

The further observation by Adrian, that the velocity of the impulse is uniform throughout the whole of a narcotised length of nerve, instead of showing progressive reduction, is additional evidence in favour of the decrementless conduction.

The most definite of the recent investigations which lent apparent support to the theory of decremental conduction were those of Lucas and subsequently of Adrian. Lucas in 1917 accepted the idea that conduction in the narcotised nerve was decremental, and based his method of estimating the magnitude of the nerve impulse in a normal nerve on this assumption, for, he says : " If we wish to measure the intensity of a nervous impulse we can set it to face a tract of nerve in which it will undergo a decrement, and determine how far it is able to travel before it is extinguished." Experiments now widely known appeared to substantiate these arguments. If impulses were just stopped by exposure of a length of say l mm. of the nerve trunk to narcosis (by dilute alcohol vapour for example), it was found that the exposure of two separate parts of the nerve, each of length $\frac{1}{2}\ l$, with a stretch of normal nerve in between, failed to arrest

the impulse: the inference led to the apparent contradiction that the impulse was all-or-none, in the normal nerve, but was decrementally conducted in the narcotised region.

Before dealing with alternative explanations of this phenomenon, we should mention the investigations of Hallowell Davis and his co-workers. Davis and Brunswick, from theoretical considerations, concluded that a valid

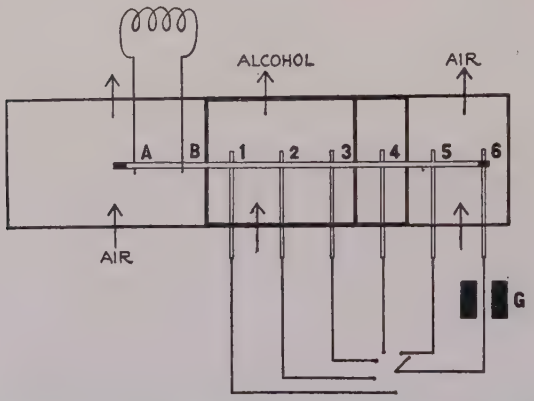

FIG. 70.—Arrangement of nerve in partitioned chamber. (Hallowell Davis and collaborators.)

objective measure of the intensity of the nerve impulse is the potential of the action current, which they measured with a string galvanometer having a slack string which behaves ballistically; they assume a constant duration of the action current in the individual fibres for short discharges. Their experiments were conducted on the excised peroneal nerve of the cat, which presents 10 to 12 cm. of uniform unbranched nerve trunk: the ends of the nerve were crushed and one electrode led off from an injured, and another from one of various uninjured points. The monophasic action potentials on maximal stimulation, remain very steady over the second to third or fourth hours

following excision. With these arrangements, Davis, Forbes, Brunswick and Hopkins stimulated the nerve at one end and studied the magnitude of the action potentials (*a*) at three points along a 45 mm. length of nerve, which could be progressively narcotised with dilute alcohol vapour, (*b*) at a point further on as the nerve passed through an air-filled chamber 13 mm. long; (*c*) at a point in a third compart-

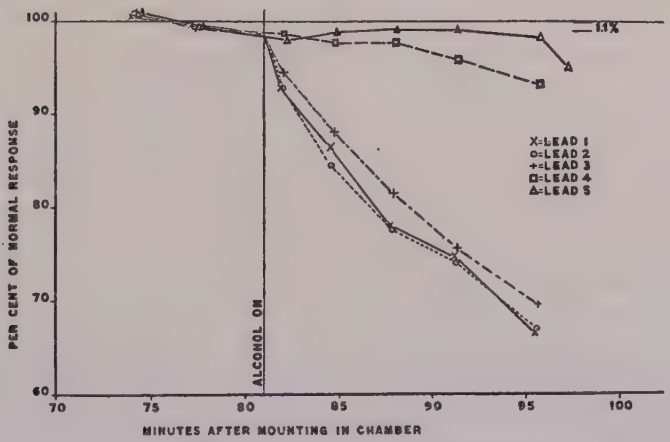

FIG. 71.—Responses of narcotised and non-narcotised portions of nerve trunk. (Davis, Forbes, Brunswick and Hopkins.)

ment filled and ventilated with moist air (normal nerve). (Fig. 70.)

The results were quite clear cut. When the nerve was first set up the response was maximal at all the leads. As soon as narcosis was begun, the responses fell off rapidly and equally at each of the three leads under (*a*), but remained for a long time constant at lead (*c*). Any change at (*b*) was due to leakage of vapour from the narcotising chamber. (Fig. 70.)

When the responses in the narcotised section of nerve had been reduced to about 70 per cent. of their initial value, however, the control in the normal nerve begins to show a

reduction: this is because the narcotic is not distributed uniformly to all fibres. These authors therefore conclude that along a relatively long stretch of evenly narcotised nerve the impulse is conducted at uniform but reduced intensity.

How then are we to explain the experiment of Adrian and Lucas referred to above? Extensive researches by Kato and his pupils at Tokyo appear to answer this question. The experiments of Adrian and Lucas had been performed on the sciatic nerve of the frog, the narcotising chambers often allowing of narcosis of only a few millimetres of nerve; repeating the experiments under these conditions, Kato obtained the same result, *i.e.*, a 3 mm. length of nerve is more resistant to narcosis than a 6 mm. length. But when the experiments are performed on *long* unbranched stretches of nerve such as are obtainable from the *n. abdominalis* or the bundle to the *flexor digitorum* group of muscles of the large Japanese toad, the results are quite different, for now it is found that the long stretch can resist narcosis for the same length of time as a short one, provided the latter is longer than 6 mm. The explanation given by Kato of the greater resistance of very short lengths of nerve is that owing to diffusion there is a gradient of narcosis from the wall of the chamber for a distance of about 3 mm., so that the full effect will never be reached at all unless the chamber is more than 6 mm. across. Hence, in two separate stretches, each of $4\frac{1}{2}$ mm., narcosis will never reach a full and uniform level, whereas in a 9 mm. stretch it would be maximal for the middle 3 mm.

Various workers have shown that when, by progressive action of a narcotic, a stretch of nerve has just failed to conduct to the muscle an impulse generated at the central (normal) end of the nerve, strong stimuli applied within the narcotised area will still provoke a contraction, especially if applied at the peripheral part of the narcotised stretch. This appeared very strongly to support the view of decre-

mental conduction. Kato has shown that it is actually due to the fact that very strong stimuli can spread far along the nerve—sometimes 30 mm., so that in the above case, such a stimulus can often affect portions of the normal nerve lying outside the chamber. When mechanical stimuli are used instead of electrical ones the response fails simultaneously to stimuli above the chamber and at all points within it.

Other evidence of a more complicated character has been added, from which we must conclude that in the nerve submitted to the action of a narcotic the impulses are of all-or-none character, as in normal nerve, but of a magnitude which diminishes with progress of narcosis.

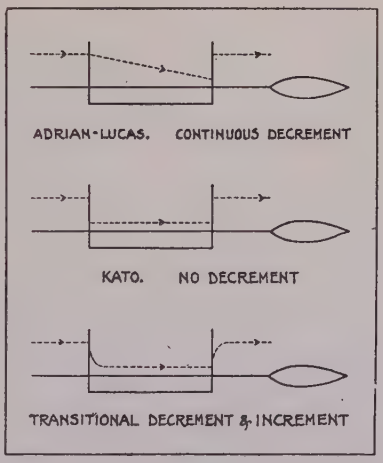

FIG. 72.—Representing the various views of the action of narcotics on nerve. (After Davis, Forbes, Brunswick and Hopkins.)

But although we must in general abandon the idea of decremental conduction, the question arises whether we may not accept the idea of a more or less gradual transition (over a short distance) from the normal to the reduced size of impulse when a region of depression is entered, and a similar graded increment when the impulse emerges into normal nerve again, as illustrated in Fig. 72. There are certain facts which suggest that this gradient might extend through about 3 mm. of nerve. Such a hypothesis indeed would seem to be a necessary consequence of the all-or-none law, for as the latter really implies that the nerve, when excited by the arrival of the impulse, liberates energy to speed this on its further journey, we cannot but suppose that

the process has a kind of momentum and so will not instantly fall to the lowest ultimate level when entering a region in which the nerve contributes at each point a smaller quantum of energy to its further propagation ; and *vice versâ*.

Cattell and Edwards, who studied the onset of block produced by the application of pressure to the nerve, found that the effect of compression of a 16 mm. length of nerve was the same as that of an 8 mm. length, but that when 4 mm. was compressed it took nearly twice as long to block 80 per cent. of the fibres. Hence they conclude that there is a transition decrement extending over not less than 4 mm. and not over 8 mm. Kato and Terruchi, however, remain firm in their rejection of even a transition decrement. It seems only reasonable, however, in spite of this, to hold to the view that some degree of transition decrement may be present as the impulse passes into altered regions of the nerve, and if this is so, it should be of importance in connection with the physiology of junctional conduction such as that at the nerve-endings in muscle or at the synapses in the central nervous system. In passing through these very short portions of junctional tissues it seems quite probable that some change, either depreciation or appreciation, in the magnitude of the transmitted impulse may occur, and that the direction and extent of this change will, as in several current theories, have important consequences in determining conduction phenomena in the central nervous system.

The Effect of Stimulation upon Nerve

(i) **The Action Current.**—*Method of Recording*—Various methods are available for the study of the action currents which accompany the propagation of the nerve impulse. Each method has its own advantages and its peculiar deficiencies.

The variations of potential in the action current are very

rapid, being normally completed in a few thousandths of a second. The moving coil or moving magnet galvanometer has considerable inertia and low sensitiveness; the string-galvanometer, a refinement of the first-named, gives the more accurate picture of rapid alterations, but such inaccuracies as it has, which are connected with its mode of movement (forced vibration with damping) are not amenable to complete correction when the acceleration of the movement of the string is rapidly changing, as in steep changes of potential. The capillary electrometer gives records much

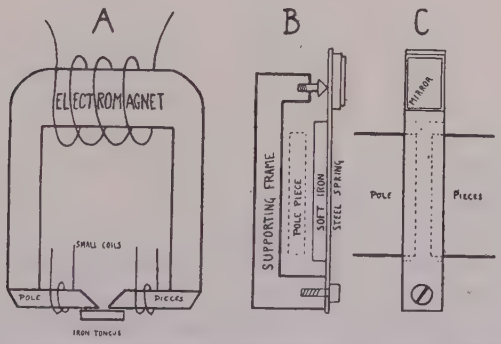

FIG. 73. Diagram of Matthews' Oscillograph. B and C show section and front view respectively of tongue and mirror. (MATTHEWS.)

further from the correct curves, though these can more fully be corrected.

It is evident that for the close examination of the action current an inertia-free method is much to be desired, and this is provided by the use of various types of oscillographs. One of the most recent, and also one of the simplest and best of these is that devised and used by Matthews (Fig. 73). This resembles a loud speaker, and has an iron tongue which follows the variations of potential, suitably enlarged by an amplifier such as the one used by Adrian. A mirror is attached to the tongue, and a beam of light reflected from this on to a photographic surface.

Another type of instrument is the cathode ray oscillograph, or Braun's tube, as modified by Johnson. The principle of this apparatus is that a stream of electrons is liberated from a hot cathode, passed through a tubular anode at a high potential and allowed to impinge on a fluorescent screen at the end of the tube, which makes the site of impact visible as a spot of light. This pencil of rays can be deflected from its straight course by the electric field provided by the action current, suitably amplified several thousand times by a thermionic valve circuit, and connected to two horizontal plates one above and one below the pencil of rays : the spot is thus caused to move vertically, and so, with a stream of repeated action currents, converts the spot into a vertical line, as in a lever writing on a stationary drum. In order to provide the other co-ordinate, represented by time, and corresponding to setting the drum in motion, the vertically spread fan of rays must be progressively pulled out in a horizontal direction by an increasing potential applied to two similar vertical plates. If the action current is repeated in sufficiently rapid succession, and the horizontal movements of the spot are synchronous and similarly repeated, a stationary wave is seen on the screen and may be traced or photographed. This apparatus gives results reliable to 0·02 σ and has been extensively used by Gasser and Erlanger, whose researches will now be briefly described.

The Action Currents.—When the action currents, led off from a nerve stimulated electrically, are recorded by the cathode ray oscillograph, it is seen that the action current commences fairly abruptly, reaches a crest without interruption and then subsides more slowly, often showing on the catacrotic limb various secondary waves, the significance of which will be discussed presently.

Gasser, Erlanger and Bishop found that the propagation of the action potential commences either immediately or in less than 0·06 σ after application of the stimulus.

The time relations of monophasic action currents in some typical nerves is shown below (Gasser and Erlanger).

	Temp. °C.	Time to Maximum.	Total Duration.	Velocity of Propagation m.p. sec.
Bull frog sciatic	11	2·03 σ	—	—
,, ,,	22·3	0·68 σ	3·26 σ	35·2
Dog phrenic .	38·7	0·69 σ	2·48 σ	52·9
,, tibial .	38	0·43 σ	1·38 σ	80·4

These results indicate that the velocity of propagation of the action potential is greater the more rapid the rise, and the briefer the duration of the disturbance, and consideration of the tracings led Gasser and Erlanger to suspect that the catacrotic waves so frequently met with in the records might be due to the presence of waves of different magnitudes and velocities which were partially fused to form a complex wave : further investigation fully confirmed this suggestion and led to most interesting results.

The Compound Nature of the Action Current.—Erlanger, Gasser and Bishop studied the form of the wave of action potential as it travelled along the nerve, and found that in many instances a wave which was apparently simple at its origin showed catacrotic waves with increasing distinctness and at increasing distances from the primary wave as it travelled along the nerve.

Some nerves, notably the phrenic nerve, show no such wave, the only change after travelling being a gradual slight slowing of all its phases and a reduction in amplitude ; owing to the corresponding increase in the duration, this reduction in amplitude is not interpreted as showing that conduction has occurred with a decrement. The explanation seems without doubt to be that the individual fibres conduct at slightly different rates, so that although the impulses in the separate axons (axon potential waves

all start off simultaneously, some of them get slightly in advance and others lag behind the majority. The amount of current and the area of the curve remain constant.

The explanation of the catacrotic waves is at once apparent; if different groups of axons conducted at widely

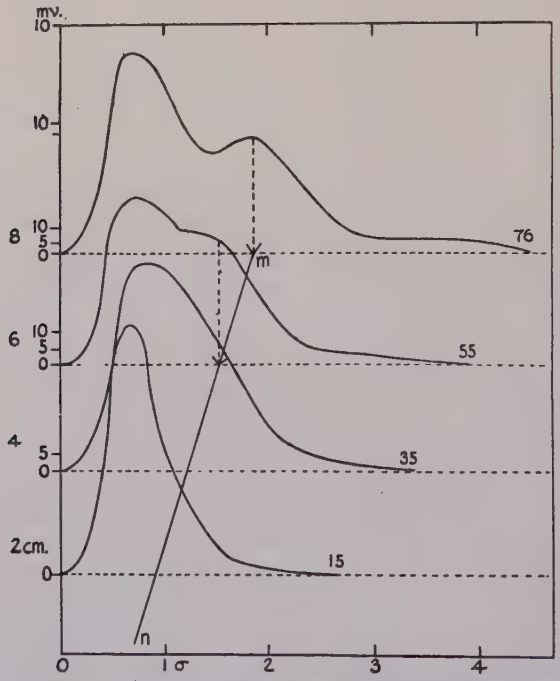

FIG. 74.—Primary and catacrotic waves led off at different points of nerve. (Gasser, Erlanger and Bishop.)

different rates, then each group would tend to separate from the other groups as the impulses progressed along the nerve, with the result that before long the slower waves of potential would separate out as catacrotic waves on the descending portion of the main waves. Gasser, Erlanger and Bishop found that this was what happened, and were able in the sciatic nerve of the bull frog to distinguish in this way

usually two, often three, and occasionally four components of the action wave. These they distinguished in the order of their velocities as the α, β, γ, and δ waves. At the origin all were fused; *e.g.*, in one experiment (Fig. 74), at 15 mm. from the stimulus only one wave was evident, at 55 mm. the β wave began to appear, at 76 mm. the γ wave; calculation showed that these waves travelled at the following velocities :—

α at 43·8 m.p. second.
β at 27·0 ,, ,,
γ at 16·2 ,, ,,

As we have already seen, the velocity of propagation is not absolutely constant from axon to axon; Erlanger, Bishop and Gasser found in fact that in the dog's phrenic, the waves, though not separable into α and β types, varied, in one case, in velocity from 60 to 41 m.p. second, and it seems indeed, usual for the speed in the fastest fibre of a group to be about 50 per cent. faster than in the slowest. In the bull frog's sciatic nerve, the extreme velocities in the α wave were, in one case, 40 and 24 m.p. second; they conclude that the usual rates of the α and β waves are the outside values for velocities of the α axon waves.

This natural grouping of velocities of impulse propagation in different axons falls into accord with the observations of Lapicque and his colleagues on the different chronaxies of fibres in a nerve trunk as discussed in Chapter X, and the measurements of axon size made by Gasser and Erlanger lend support to the suggestion that the differing velocities of propagation are associated with different diameters of nerve fibre. The phrenic nerve of the dog consists of motor and sensory fibres in the neighbourhood of 8 μ in diameter, the range being from 5 to 12 μ, and as already stated the phrenic gives a simple action potential curve. The motor root of the ninth nerve of the bull frog is of fairly uniform histological structure with the bulk of its fibres of about 15 μ

in diameter: the sensory root on the other hand, is very heterogeneous, containing fibres from 20 μ to 3 μ, the peak of the distribution being at about 8·5 μ. These measurements explain the observation of Erlanger, Bishop and Gasser, that when the sciatic nerve trunk is stimulated, the action potential wave passing into the anterior root is of simple form, whereas that passing into the posterior root shows (sensory) waves which contain α, β and γ components. It seems clear from these observations that the axon diameter is the factor which determines the velocity of propagation of the action potential and therefore presumably of the nerve impulse. There is an approximately linear relationship between the diameter of fibre and velocity of propagation.

In the frog the α waves travel at about 40 m.p. second in fibres of about 15 μ diameter, the β waves at about 25 m.p. second in fibres about 8 μ, and the slower waves in fibres of 5 μ or less. In two different species of frog (green frog and bull frog) it was found that differences in rate of conduction of the main wave were associated with differences in the size of the majority of the fibres, the axons of the green frog sciatic being smaller and conducting more slowly than those of the bull frog. In the chordo-lingual nerve of the dog the action current was found to show a small secondary wave which was traced into the chorda tympani, whose fibres have a diameter around 4 μ and conduct at 36·4 m.p. second, while the lingual fibres were much larger and conducted more quickly.

It was also found that the α wave could be generated alone in a mixed nerve by such adjustment of the stimulus that it failed to excite the slower fibres which have a longer chronaxie; similarly by appropriate stimulation the pure β wave could be excited. There are similar relationships between the absolute refractory periods, *e.g.*, 1·2 σ for α, 1·68 σ for β and 3·13 σ for the γ fibres in a particular case.

That these different types of action potential are asso-

ciated with different functions is clear from their histological and anatomical relationships. It is suggested that motor impulses to muscles, muscle sensory impressions and afferent depressor impulses belong to the α class; the β sensory impressions evoke respiratory reflexes and the γ pressor reflexes: a further analysis of these functional aspects of the problem should prove most valuable.

In addition to the α, β and γ waves, Gasser and Erlanger have recently (1929) described much more slowly-moving potential waves, which appear when very strong stimuli are applied. Calling the confluent α, β and γ waves hitherto described the A type, the new and slowly-travelling types may be called B and C. The relative average velocities are, in m.p. sec.:

	Bull Frog (Room temp.)	Green Frog. (Room temp.)	Dog. (Body temp.)
A. . .	40–10	35–8	80–30
B. . .	5–2	5–2	17–11
C. . .	0·7–0·5	0·7–0·5	1·5–0·8

There are indications that the B and C waves are associated with the presence of non-medullated fibres, and these do not show the same relation between diameter and velocity of propagation as are seen in the α, β and γ components of the A type.

Recent observations by Matthews, using his oscillograph, give grounds for supposing that the electrical variations, set up from different receptors, are not necessarily absolutely identical. He employed a preparation from the frog, in which skin and muscle are supplied by branches from a common nerve trunk. The skin was stimulated by pressure, and the muscle proprioceptors by stretching, and the resultant electrical potential changes in the common nerve were recorded. It was found that when the muscular receptors were stimulated, the potential changes were not identical with those when a stimulus was applied to the skin. (Different kinds of skin stimuli, however, *e.g.*, pressure or pinpricks, gave indistinguishable, if not identical, potential

waves.) The following data from a typical experiment illustrate the differences (at 10·5° C.) :—

	Cutaneous impulses.	Muscle proprioceptor impulses.
Conduction rate m./sec. . .	8·0	11·5
Time in σ to max. potential .	1·8	1·5
Total duration, in σ . .	5·0	4·3
Relative maximal potentials .	80	100
Relative potential gradients .	62	100

It is thus seen that the skin " impulses " have a slower conduction rate, a smaller potential, slower rise, and a longer total duration than the impulses from the muscles. In addition to these impulses, which were approximately constant in character, there were seen, especially on skin stimulation, some small potential waves travelling at about half the rate, and also some impulses of twice the amplitude, but otherwise identical with the principal waves. The slow waves were unexplained, but may be of the same nature as those described by Gasser and Erlanger as the γ waves. The double-sized impulses were believed to be due to unexplained synchronous response from two end organs.

On electrical stimulation of the separate muscular and cutaneous branches of this preparation, it was found that the muscular branch yielded a simple wave of α type, from which it was concluded that both motor and proprioceptive impulses may contribute to the formation of the α wave. Electrical stimulation of the cutaneous branch, on the other hand, produced a compound electrical disturbance, of which the first ripple resembles a wave slightly faster than the α wave, and called the pre-alpha by Gasser and Erlanger, while the other is a typical β wave. It was found that the β wave travelled at the same rate as the wave which resulted from cutaneous stimulation. Sometimes also there was a

γ wave. The diameter of the preponderating groups of nerve fibres in the muscular branch (6–8μ), and in the cutaneous branch (under 6μ), was in agreement with the observed rates of conduction.

These experiments therefore suggest, though they do not exactly prove, that the different qualities of sensations from different end-organs may be due, not only to a different destination of the fibres in the central nervous system, but also to small differences in the gradient, duration or magnitude of the impulses. It is possible that these differences in type of impulse may play an important part in deciding the path which might be taken by these impulses in the central nervous system, and the types of reflex to which their arrival there might give rise.

(ii.) **Depolarisation.**—This phenomenon is shown most clearly by non-medullated nerve, and has of recent years been studied by Levin and by Furusawa. Using the nerves of crustaceans, Levin found that on repeated stimulation these showed definite evidence of fatigue, as indicated by the great reduction of the action currents. The nerve thus fatigued will rapidly recover if kept in air or oxygen. The fatigue of the nerve is accompanied by a steady reduction in the injury current, so that the current of action, which in the injured nerve is monophasic, and is a return towards zero potential (" negative variation of current of rest "), becomes smaller, and finally disappears altogether.

This reduction in the monophasic action potential was called by Levin " the retention of the action current," but has been more clearly explained by Furusawa. Modern views of the electro-physiology of nerve regard the phenomena as connected with polarisation effects at membranes ; in this condition we have ions of one sign on the outside of the membrane, and those of the opposite sign on the inside. This is the resting condition of the tissue, and the maintenance of this state depends on the continuous expenditure of energy, *i.e.*, on combustive processes, even when the

nerve is at rest. When we lead off from an uninjured and an injured surface, we get a potential difference due to this polarisation, the injured surface giving us the charge of the inner surface, and the intact surface that of the outer one. When a nerve impulse passes any point, we may suppose that this polarisation is reduced, so that the injury current between an active point and an injured point becomes less ; it may even fall to zero. If oxygen is present, this depolarisation is quickly recovered from, with the expenditure of energy, though in non-medullated nerve this does not happen very rapidly. Hence we get the phenomenon of an apparent lingering of the action current, which is really due to persisting depolarisation of the membranes involved in the action current response. Even at rest, in absence of oxygen, the potential of the injury current falls, and, on readmission of oxygen, recovers.

(iii.) **The Refractory Period.**—Under normal conditions nervous impulses often follow one another in a train in rapid succession along the nerve fibre. All except the first of these must pass through tissue which is modified by the passage of previous impulses.

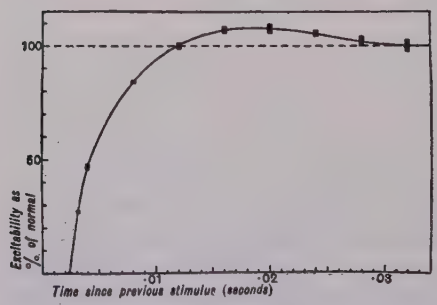

FIG. 75.—Recovery of excitability of frog's nerve. (Adrian and Lucas.)

After an impulse has passed along a nerve fibre this is rendered quite inexcitable and non-conductive for a short period, called the *absolute refractory period.* During this period the nerve fails to conduct an impulse, and stimulation leads neither to any electrical response nor to contraction of the muscle supplied by the nerve. Its duration varies usually from 1 to 3 σ, and is related to the phenomena of general ex-

citability of the fibre ; the larger fibres of short chronaxie and high propagation velocity have a shorter refractory period than the slower fibres. But the refractory period is not coterminous with the action potential wave, since the latter is over in about 3 σ, whereas the absolute refractory period is followed by a *relative refractory period* of about 10 σ duration, in which the excitability is depressed but gradually recovering ; this again is, under particular conditions, followed by the *supernormal* phase, in which for a time the fibre shows augmented excitability (Fig. 75).

The refractory period is shortened by rise of temperature, the temperature coefficient determined by Adrian being about 3, whereas the temperature coefficient for the velocity of conduction is in the neighbourhood of 2. The occurrence of the refractory period signifies that as a result of the passage of an impulse some change occurs in the fibre from which it must recover before a second impulse can pass or be generated. This change might be a chemical one, such as the exhaustion of some material, or a physical one, *e.g.*, a displacement of ions. The suggestion from the determinations of temperature coefficients is that the velocity of conduction might be, and the duration of the refractory period most probably is, ultimately dependent upon the occurrence of some chemical reaction or reactions. This is strengthened by the observation of Field and Brücke, that the refractory period of the frog's sciatic may be increased to as much as 9 σ after ten minutes of faradic excitation, and slowly returns to normal with rest.

During the relative refractory phase the impulse was shown by Adrian and Lucas to be conducted without any decrement, though its intensity, as gauged by the magnitude of the electric response and in other ways, is reduced. Gasser and Erlanger, in studying the electric response, found that the time relations of a second response during the relative refractory period were the same as those of a first response, but that the second response was propagated more

slowly. By lagging behind its predecessor, however, the impulse is continually passing into nerve which has had more and more time to recover from the refractory state left in the wake of the first impulse, so that its velocity rises exponentially at first rapidly, then more slowly until at length it nearly reaches its normal velocity ; in the bull frog's nerve the velocity has reached a large fraction of the normal when about 10 cm. of nerve has been traversed. But a much greater distance would have to be travelled before the impulse passed into completely normal nerve, in fact, not until it lagged so far behind as to allow the nerve nearly $\frac{1}{100}$ of a second between the impulses. As the velocity increases the size of the impulse also recovers towards the normal.

Adrian and Lucas found that the second impulse was unable to pass a region of moderate narcosis passable by the first impulse ; if we accept the region of narcosis as a region of impaired, though not of decremental, conduction, we might explain this failure to pass as due to the prolongation of the refractory period in the narcotised region ; this explanation would not, however, be universally approved.

The supernormal phase in excitability and conductivity following the relative refractory period is only seen when the nerve is immersed in solutions of slightly acid reaction, and Adrian points out that this is comparable with the staircase phenomenon in cardiac muscle, which is also only seen when the perfusion fluid is slightly acid or neutral. He suggests as an explanation of the phenomenon that excitability and conductivity are greatest when the colloids of the membranes concerned are uncharged, and that activity produces a negative change ; as the tissue recovers it eventually passes through an uncharged phase if the surroundings are slightly acid, since these alone would impart a slight positive charge.

The impulse of the supernormal phase has the same time relations as the normal impulse, though the electric potential of the response is perhaps amplified. Cooper finds that the

velocity of the impulse in the supernormal phase is not increased.

The existence of the supernormal phase may perhaps prove of value in explaining the facts of summation in the nervous system, although it is not yet clear quite how it would be applied. If we accept the response of the individual fibre as being all-or-none, and agree that this is followed by a refractory period, then any augmentation by further stimuli of an impulse once commenced must be impossible. Summation can be considered only as the adding together of inadequate stimuli until these reach the threshhold of excitation. Many inquirers have sought to know what is the nature of the summation which occurs in nerve centres as a result of the arrival of repeated nerve impulses at the synapse. It is conceivable that the supernormal phase may be engendered at a synaptic junction, at which, by reason of a transition decrement, a first impulse has been quenched.

The converse problem is seen with what is called the *Wedensky inhibition*, to which appeal has often been made in attempts to explain central inhibition. The Wedensky inhibition is seen in a partially fatigued or narcotised nerve muscle preparation. On applying rapidly repeated stimuli to the nerve, the muscle gives one twitch and then relaxes completely ; it is believed that only the first impulse generated in the nerve is passed on to the muscle, the succeeding ones being blocked in some way, as to the mechanism of which opinions differ. A probable explanation is provided by Forbes, Ray and Griffith, who suppose that whereas the full-sized first impulse passes through to the muscle, the undersized impulses following in relative refractory periods are extinguished. If this explanation applies to the synapses in the central nervous system as well as to the neuro-muscular junctions, then it would appear that summation and inhibition might well be explained on the lines given above. There are, however, many other theories which attempt to explain these phenomena.

(iv.) **The Heat Production of Nerve.**—Until quite recently all the available evidence indicated that the nerve during its activity either produced no heat at all or released it in such small amounts that it escaped detection by any methods at our disposal. This, together with the fact that medullated nerve, within the ordinary duration of an experiment, is incapable of fatigue, gave grounds for the belief that the propagation of the nervous impulse was of a physical nature. Yet it had long been known that in the absence of oxygen, nerve soon lost both its excitability and its conductivity, often to regain these when oxygen was readmitted. It was supposed, therefore, that although no chemical reactions were involved in the actual transmission of nervous impulses, chemical reactions into which oxygen entered were essential for the maintenance of the excitable condition. The action of narcotics, and the facts connected with the refractory period were similarly regarded as not directly connected with chemical phenomena. Attempts to demonstrate increases in metabolism of the nerve during excitation had, until quite recently, given equivocal results.

In 1926, Downing, Gerard and Hill, by the use of thermopiles of great sensitivity and a new method of detection, were able to show that small amounts of heat are produced on the excitation of a nerve, and thus opened up a new method for the investigation of nervous conduction.

In the apparatus which they employed nerves were placed in a groove in contact with three or more delicate thermopiles, each thermopile containing about 100 couples of constantan-silver made by the electro-plating method. The distal end of the nerve was stimulated by induction shocks; between the stimulating electrodes and the thermopile the nerve was laid on a grid of silver wire which absorbed the heat produced by the electrical current at the site of stimulation. The heat liberated at the thermopile junctions was therefore due to the passage of nervous impulses along the portion of nerve in contact with them. The thermopile and the stimulating electrodes were placed in a glass container which was immersed in well-stirred water in a double-walled vacuum flask to keep its temperature constant. The current produced in the thermopile as a result of the warming-up

of the nerve was led to a galvanometer working on the thermal relay principle, which is as follows: The current from the thermopile passed to a delicate moving coil galvanometer from the mirror of which a beam of light was reflected. This beam fell halfway across the slit of a radiation thermopile, and by warming the junctions, set up a constant current through a second delicate galvanometer with which it was connected. When the mirror of the first galvanometer moved slightly, this caused either more or less of the reflected heat to fall on the radiation thermopile and so produced alterations in the current passing through the second galvanometer. The result was an arrangement so sensitive that a current of 2×10^{-12} amps. from the nerve thermopile produced 1 mm. deflection at a distance of 1 metre. The apparatus was calibrated by generating heat in the nerves with an alternating current too weak to stimulate or by passing stronger currents through the dead nerve.

When a nerve is at rest, apparently very small amounts of heat are produced. These cannot be estimated directly with much accuracy, but the amount found, viz., about 2×10^{-5} calories per gram per second, appears, from observations of the oxygen usage (see later) to be of the correct order.

When the nerve is stimulated, many induction shocks must be given, even with this delicate arrangement, in order to produce any detectable amount of heat. When at 15° C. 280 shocks per second were given the heat generated was, at the commencement of stimulation, $7{\cdot}6 \times 10^{-6}$ calories per gram of nerve per second of stimulation. This is followed after the stimulation by a small but very prolonged heat production lasting for about ten minutes, the total amount of excess heat produced during and after one second of stimulation being $6{\cdot}9 \times 10^{-5}$ calories; in other words, the nerve liberates a small amount of heat during the actual stimulation, but this is only about 11 per cent. of the total heat produced during stimulation plus its after effects.

These findings were confirmed by Gerard, Hill and Zotterman, who calculate that the initial heat for one impulse through 1 gram of nerve amounts to 10^{-7} calories,

and the delayed heat to $8{\cdot}5 \times 10^{-7}$ calories. The corresponding figures for a single impulse traversing 1 cm. of a single fibre are probably about 10^{-13} calories for the initial heat and $8{\cdot}5 \times 10^{-13}$ calories for the delayed heat. On theoretical grounds it is believed that the initial heat production is very abrupt and is probably over in about 4 σ, that is to say, it is probably of the same order of duration as the action potential wave. The delayed heat indicates some kind of recovery process which certainly extends far beyond the duration of the refractory period. It is suggested by Gerard

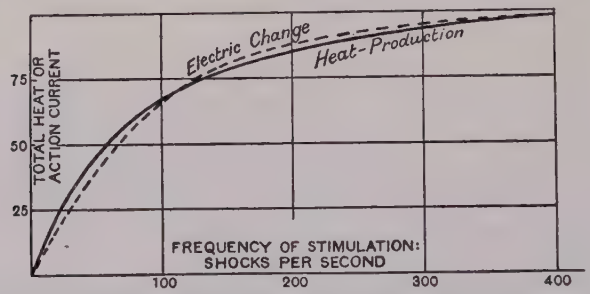

FIG. 76.—Relation between total heat per second (full curve) or total action current per second (dotted curve), and frequency of stimulation, data at 400 stimuli per second being taken as 100. (Gerard, Hill and Zotterman.)

that in the recovery process some substance formed during activity is being slowly removed, entering as a single component into some reaction.

In the non-medullated nerve of the crab Hill finds that only about 2 per cent. of the heat is generated during activity, the remaining 98 per cent. being emitted after activity. This recovery heat production is 20 to 40 times as great as in the frog's medullated nerve, and occupies about half an hour. It is probably in great part oxidative in nature, and no doubt represents the energy involved in restoring the state of polarisation of membranes after activity has caused them to be depolarised. The relations are quantitatively if not qualitatively different in medullated nerve. For instance, the non-medullated nerve gives at least $2{\cdot}5 \times 10^{-3}$ cal. total heat per gram per second of maximal stimulation, which is about thirty-three times as much as for frog's medullated nerve.

The effect of frequency of stimulation on the heat production of nerve has been investigated by Gerard, Hill and Zotterman, who find that as the frequency of stimulation is raised the total heat production is also raised, but not in proportion to the number of shocks ; that is to say, that the amount of heat generated per impulse becomes less as the impulses follow one another in more rapid succession. The following abstract from their data and Fig. 76 illustrate this :—

Absolute Values of the Heat per Impulse at Different Frequencies, reckoned per Gram of Nerve (Gerard, Hill and Zotterman).

Frequency (Shocks per sec.).	Interval between Shocks (σ).	Observed Initial Heat 10^{-3} cal.	Calculated Total Heat 10^{-3} cal.
400	2·5	2·03	18·4
280	3·37	2·71	24·6
200	5·00	3·47	31·5
100	10·0	5·53	50·2
20	50·0	9·49	86·1
0	∞	10·32	93·7

The figures in the lowest line refer to the data for a single isolated impulse in the nerve and were extrapolated from the other observations. They show that for one isolated impulse there is an initial rise of temperature of almost exactly 1/10,000,000 of a degree centigrade during stimulation. In muscle giving a single twitch the initial rise of temperature is about 30,000 times as great. The table shows that the initial heat per impulse when stimulated with the frequency of 280 shocks per second (the usual rate employed in most of the experiments) is only about one-quarter or one-fifth as great as that when slow shocks are given. The values given for total heat were calculated on the assumption that the relation between initial heat and delayed heat remains the same, whether the former is large or small.

These investigators determined also the total action current produced in the nerve when stimulated at different frequencies, and found that it gave a curve very similar to that for the initial heat production (see Fig. 76). These findings are of considerable interest in connection with the data discussed above on the relative refractory period. The relative refractory period lasts from 10 σ to 20 σ, and when stimuli follow one another at shorter intervals than this, *i.e.*, frequencies greater than 50 to 100 shocks per second, it is seen that each impulse produces less heat and a smaller

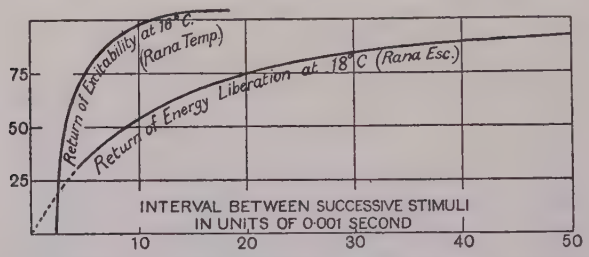

FIG. 77.—Return of energy liberation and return of excitability: energy liberation = heat per impulse expressed as a function of the interval between successive stimuli. Return of excitability derived from data by Adrian by measurement of least interval for muscular summation. (Gerard, Hill and Zotterman.)

action potential, both curves bending over at about this frequency. We may suppose that after an impulse has passed, the nerve is for some 10 σ to 20 σ in a relative refractory condition, liberating a smaller amount of energy and giving a smaller action potential wave until this relative refractory period has been passed.

It is possible from the data provided by stimulation at different frequencies to plot a curve relating the average heat production per impulse to the interval between successive stimuli and thus to ascertain the extent to which the capacity of heat production is able to return towards the normal between stimuli. When such a curve for return of energy liberation is compared with the return of excitability as determined by the influence of a second stimulus following

at measured intervals from the first (relative refractory period), the result (Fig. 77) indicates that excitability is recovered in about 10 σ, whereas the energy liberation with intervals of 10 σ between stimuli is only about 60 per cent. of the full strength. Even after 50 σ, as the figure shows, the energy liberation is not quite back to normal. The explanation for this probably is that in this method we are dealing with fibres which recover at different rates, proportionately, no doubt, to their different dimensions, and differences in their physiological characters, whereas in the determination of return of excitability the recovery of the fastest fibres only is measured.

From these data we may picture the events associated with the passage of a nervous impulse to be somewhat as follows : accompanying the actual passage of the impulse there is a small and very rapid initial liberation of heat, occupying only a few σ. During the initial liberation of heat, or at all events, during its early stages, the nerve is completely inexcitable, *i.e.*, it is totally unable to liberate any energy on receipt of a second stimulus : hence the refractory period. This is followed by a recovery process comparable with relaxation in the muscle and taking in the slowest fibres 50 σ or more to complete. During the period of recovery the excitability of the nerve increases, at first rapidly during the relative refractory phase, and then more slowly. At the close of this recovery period the nerve is capable of generating a full-sized impulse accompanied by the full heat liberation and action potential characteristic of its fresh condition. This is followed by a third phase, lasting at room temperature for ten minutes, during which about eight times as much heat is liberated as during the first two phases. This corresponds with the restitution period of muscle.

It might have been anticipated by comparison with what happens in muscle that the initial and recovery processes would be independent of the presence of oxygen, but that

the long-lasting restitution process depended on the presence of oxygen. Gerard, however, finds that this anticipated parallel cannot be demonstrated. When oxygen is withheld from the nerve there is (unlike muscle) a progressive fall in initial heat and activity leading to its complete extinction after two or three hours of oxygen lack : together with this the delayed heat is not abolished, as it is in muscle, but falls off proportionately to the initial heat. There is also progressive fall and final extinction of the average action potential, though this does not fall parallel with the heat production. The falling off in initial and delayed heat production is due partly to individual fibres becoming completely inactive and partly to decreased heat production in all the fibres. This ends with the death of the fibres if oxygen lack is continued long enough.

When oxygen is readmitted to the nerve the capacity to produce heat on stimulation returns, but requires an hour or two for complete recovery. The action potential returns more promptly to the normal.

It is possible that the continuation, in the absence of oxygen, of delayed heat production in the same ratio to initial heat production as when oxygen was present may be due to the fact that the nerve is carrying out its oxidations anaerobically by utilising some hydrogen acceptor. The smallness of the metabolism of nerve makes the decision of this question rather difficult. As stated by Hill, the stimulation by 280 shocks per second of a muscle for one second produces as much heat as would be generated by similar stimulation of the same weight of medullated nerve for two hours, so that the relative unfatigability of nerve is readily understood.

(v.) **The Metabolism of Nerve.**—Nerve continually uses oxygen and produces carbon-dioxide ; it also produces small quantities of ammonia. Many attempts have been made to determine the oxygen utilisation and carbon-dioxide production of nerve, and to ascertain whether or

not these are increased during activity. From the data obtained from heat production it is possible to calculate how much the oxygen utilisation should increase during stimulation. We have seen that the excess heat during and after one second of stimulation is $6{\cdot}9 \times 10^{-5}$ calories per gram. Since one calorie corresponds to about 0·2 c.c. of oxygen, this heat production should represent increased oxygen utilisation of about 48 c.mm. of oxygen per gram of nerve per hour of stimulation. Similarly, the resting heat production (approximately estimated at 2×10^{-5} calories per gram per second) should give a resting oxygen consumption of 14 c.mm. per gram per hour. The quantities of carbon dioxide should be equal to, or slightly less than, those of oxygen.

The results actually obtained by chemical methods have varied very widely. The resting oxygen or carbon dioxide figures obtained have varied from 0·002 mg. to 0·06 mg. carbon dioxide per gram per minute, *i.e.*, from 60 c.mm. to 1800 c.mm. per gram per hour, values which are all of them far in excess of the resting heat production, as estimated by Gerard. Absolute increases during activity found by chemical methods have, however, agreed much more closely with the excess heat production during and after stimulation. Thus, Parker finds an increase on stimulation of about 29 c.mm. of carbon dioxide per hour, and Fenn 22 c.mm.

Gerard has recently investigated this aspect of the subject and obtained figures which much more closely correspond with the heat measurement, namely, 16 c.mm. of oxygen per gram per hour for resting frog's nerve, and 61 c.mm. per gram per hour during stimulation. He finds that the oxygen consumption remains constant for many hours, that the temperature coefficient at rest is 2·2 between 15° and 25° C. The oxygen utilisation is diminished, though not entirely abolished, by cyanides. Cutting, crushing, or the action of chloroform temporarily increase the oxygen utilisation. The respiratory quotient at rest is 0·77.

When the nerve was stimulated continuously with 280 shocks per second its oxygen metabolism was about double that during rest, namely, it increased about 18 c.mm. per gram per hour. Intermittent stimulations applied for twenty-two seconds every four minutes increased the oxygen utilisation more than three times as much as continuous stimulation, namely, by about 60 c.mm. The explanation of this is similar to the explanation of the smaller heat production per stimulus for rapid as compared with slow rates of stimulation, and it is suggested that the nerve strikes an equilibrium when it is repeatedly stimulated ; the energy liberation per impulse falling when the stimuli follow one another in rapid succession for relatively long periods.

The excess oxygen utilisation does not commence or cease abruptly with the beginning and end of the stimulation, but rises for at least fifteen minutes after commencement of stimulation and slowly falls during fifteen minutes after its cessation before reaching the resting level. Here, again, the results correspond very closely with the delayed heat formation seen in the thermal investigations. The temperature coefficient during activity seems to be the same as during rest, but the respiratory quotient of the excess metabolism is 0·97. It might be supposed that this figure indicates a preferential utilisation of carbohydrates, but about 0·45 mg. per cent. of extra ammonia is formed in activity, which amount, if derived from protein, would mean that only protein need be oxidised to account for the oxygen consumed, and protein, if burnt to CO_2, water and ammonia, would give a respiratory quotient of 0·95. The nature of the material consumed therefore is not certain.

There are further discrepancies between the metabolism of nerve and of muscle. In the case of muscle, the ratio of initial to delayed heat is as 1 : 1, whereas in nerve it is 1 : 9. This might be due to the fact that if lactic acid is produced in nerve as it is in muscle, it is all oxidised instead of being largely restored to glycogen.

Investigation of the action of oxygen lack on nerve provides another means of approach to the problem. The loss of conductivity happens more rapidly during stimulation in nitrogen than during rest. On readmission of oxygen the nerve recovers its excitability and conductivity, but not fully unless it is also washed in Ringer's solution; even washing the asphyxiated nerve with oxygen-free Ringer's solution to some extent restores it. We may interpret these results as suggesting that during anaerobiosis toxic products accumulate, and that these can, to some extent, be removed by washing, and to some extent by the presence of oxygen. The nerve is capable of carrying out its functions perfectly well in the absence of oxygen until these products have accumulated to a sufficient concentration.

Gerard has investigated by chemical methods the effect of oxygen lack, and together with Meyerhof has studied the lactic acid formation in anaerobiosis. When the nerve is kept in nitrogen it develops an oxygen debt, so that, when subsequently supplied with oxygen, it takes up, for about an hour, more than its previous resting utilisation. During its stay in nitrogen, carbon dioxide is not only expelled from pre-formed combinations, but the nerve also actually produces about one-tenth as much carbon dioxide as it would have done if kept in oxygen. This, together with the other facts of oxygen usage and carbon dioxide production gives support for the belief, referred to above, that the nerve is able by the employment of hydrogen acceptors to carry out the oxidations upon which its activity in the complete absence of oxygen is dependent.

When nerves are kept in nitrogen, Gerard and Meyerhof find that lactic acid slowly begins to accumulate and reaches a maximum rate of about 7 mg. per cent. per hour after two or three hours; the formation then gradually ceases. If glucose is added to the Ringer's solution in which the nerves are kept, the maximum rate of lactic acid formation continues even for many days. In the absence of added glucose

the maximum concentration of lactic acid reached is about 0·1 per cent., and this happens in about thirty hours at 15° C. This corresponds with the amount of carbohydrate present in the fresh nerve. The conditions of lactic acid formation, therefore, are similar to those in muscle so long as the tissue remains at rest, and it would seem that the resting metabolism of the nerve might reasonably be attributed to a utilisation of carbohydrate through the intermediation of lactic acid.

When the nerve is stimulated in nitrogen, however, the rate of lactic acid formation is not increased. Hence, one must assume either that carbohydrate is not utilised in the energy changes associated with conduction, or else that, as suggested from the delayed heat measurements, all the lactic acid produced in activity is anaerobically and rapidly oxidised away. Since this would suggest that the metabolism of activity is qualitatively different from that during rest, it does not seem to be very probable.

Holmes and Gerard have carried out experiments on mammalian nerve, which showed that lactic acid was produced when the nerve was kept in nitrogen; this they showed to arise from carbohydrate, partly from glycogen, and partly from "free sugar." When in oxygen there was no production of lactic acid, nor removal of pre-formed asphyxial lactic acid, though there was utilisation of carbohydrate. If this lost carbohydrate were removed by oxidation, their results suggest that about 60 per cent. of the metabolism of resting nerve may be accounted for in that way. These changes in the resting nerve were not altered by stimulation; in other words, the mechanism of the energy liberation in nerve is quite different from that in muscle. Their investigations also indicate that phosphorus probably plays no important part in the metabolic changes of nerve. Ashford and Holmes found that in the grey matter of brain tissue, free glucose is an important, and glycogen a minor, source of lactic acid. In the former, phosphates also

play no part, but the lactic acid formation from glycogen does depend on the availability of phosphate.

The oxygen utilisation of crab's non-medullated nerve has been studied by Meyerhof and Schulz: they found it to be about four times as great as for the frog's sciatic. The oxygen utilisation was increased after stimulation to about nine times the resting value, but even then was very many times less than would be calculated from Hill's thermal observations.

Humoral Views of Nervous Action.—These can be only very briefly mentioned.

It was shown by Loewi that when a perfused frog's heart is arrested by vagus stimulation, the perfusate, if circulated through a second heart, will inhibit this too; further, it will cause contraction of the frog's stomach. It has been shown that the active substance responsible for these effects is dialysable and heat stable, is soluble in alcohol but not in ether, and appears to contain an ester of choline.

Similarly, when the accelerans of the first heart is stimulated both hearts show augmentation, and the stomach relaxation.

The question arises, how far we may regard this type of effect as general, and consider that nervous impulses produce their influence on their effectors by the liberation of small quantities of potent materials at their endings. Vagus excitation, according to this view, liberates a substance resembling an ester of choline, and these esters are known to produce effects very similar to that of vagus excitation: sympathetic excitation would liberate at the nerve terminals a substance resembling or identical with adrenaline, the actions of which we know closely to resemble sympathetic excitation. The development of the medulla of the suprarenal from a sympathetic ganglion encourages this view of the interrelation between sympathetic excitation and adrenaline liberation.

An attractive theory of central excitation and inhibition

along these lines has recently been put forward by Sherrington and a brief account of it is given by Fulton (*q.v.*).

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CHAPTER X

EXCITABILITY AND CHRONAXIE

A VERY cursory examination of the properties of living tissues demonstrates that their excitabilities show remarkable differences from one tissue to another, and sometimes from time to time in the same tissue.

Some tissues require on galvanic stimulation a much stronger current than others, or, on faradic stimulation, a closer coupling of the secondary to the primary coil, which implies a stronger induced current. Again, some tissues respond to galvanic but not to faradic stimulation, or *vice versâ*. It is clear that comparison of excitabilities in different tissues is unsatisfactory, if at all possible, on such indications as these. For example, a striated muscle which has been denervated for some weeks, if tested by faradic stimuli, would be reported to have lost its excitability completely, whereas if examined by galvanic currents it would be found to have retained quite definite though quantitatively altered excitability.

In view of the fact that there is no fundamental qualitative difference between galvanic and faradic currents, such a statement as " excitable to faradic, inexcitable to galvanic electricity," so often met with, is a purely empirical one which refers to the conditions obtaining in a particular experiment and which has no value whatever as a generalisation on which to base our views of the nature of excitability. In general terms it might be said that the excitabilities of two tissues stand to one another inversely as the strengths of minimal stimuli necessary to excite. Stimuli of various kinds may be employed, but electrical stimuli are the best

and most practicable; this appears to be because the fundamental phenomena of excitation are themselves of an electrical nature, and it is no doubt on this account that electrical stimulation can be effected by the expenditure of surprisingly small amounts of energy.

Galvanic Stimulation.—Dealing with galvanic stimulation as more readily analysable than faradic, it is found that the following quantities must be taken into account in studying excitability :—

(*a*) The *current density*, or strength of current relative to the sectional area of the tissue through which it flows. This should strictly be expressed in ampères or milliampères per square centimetre or other convenient unit of cross-sectional area, or in other similar terms, if it is desired to express current density directly. But since in many cases we do not know through what sectional area the current actually flows, we must often be content with knowing either the *current strength* (C) in ampères or milliampères which is employed under given conditions, or else the *voltage* (V) required to stimulate, to which under identical conditions, the current strength and density are proportional, since from Ohm's law $C = \frac{V}{R}$, it follows that if the resistance of the circuit plus tissues is the same, C will vary directly with V.

(*b*) The *duration of the flow* of the exciting current. It has been found, on the one hand, that a current that will just excite when of a particular duration, fails to do so when its duration is reduced below a certain level; on the other hand, (for a given current) beyond a particular duration, differing for each tissue, further continuation of the current produces no further effect. Hence as a first approximation, when we combine the current strength and its duration, we may say that a certain minimal *quantity* of electricity is necessary for excitation. Quantity is the product (C*t*) of current by time.

An elementary illustration, given by Lapicque, will serve

to show the importance of the time factor in excitation. A liquid potentiometer is used consisting of a groove about 1 sq. cm. in cross section, cut in a length of paraffined wood and filled with zinc sulphate solution. Into it dip two plates of amalgamated zinc A and B (Fig. 78), placed about 1 metre apart and connected with the source of current; two other similar plates also dip into the gutter; O is stationary and near to A, while C can be moved freely in and out of the solution, thus making and breaking contact. The electrodes EE are placed in contact with the tissue. The strength of

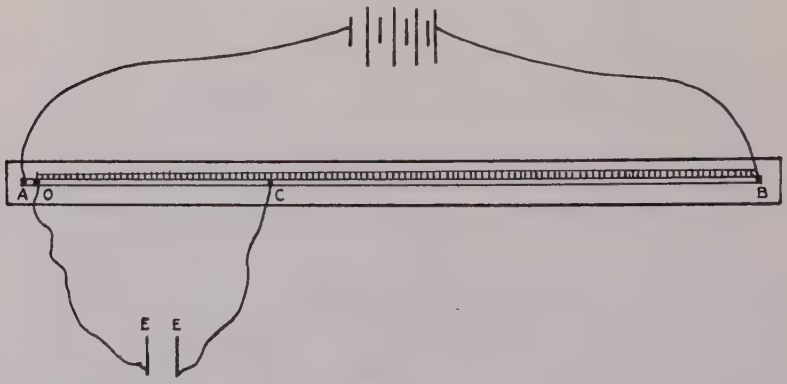

Fig. 78.

current flowing through the tissue is proportional to the distance of C from O. If a strip of plain muscle from the frog's stomach be placed in contact with the electrodes and C be plunged into the groove at intervals of a few seconds, further and further from O at each immersion, a point, the *threshold* will be reached, at which each prolonged immersion is followed by the slow contraction which characterises plain muscle. The distance OC at which this occurs (say 10 cm.) is noted. Now C is withdrawn and dipped as quickly as possible in and out of the groove at the same point, giving stimuli of the order of 1/10 second; no contraction will result. If the distance OC is made greater, however (say

70 cm.), so as to increase the potential tapped off, even this brief duration will stimulate.

(*c*) The *rate of change* of the current strength must also be taken into account. In the above paragraphs (*a*) and (*b*), it has been assumed that the current was established and stopped instantaneously ("rectilinear waves") as in Fig. 79 (A), whereas under certain circumstances a current might fluctuate by a slope, *e.g.*, as shown in Fig. 79 (B) (either linear or exponential), and these fluctuations would need to be taken into account.

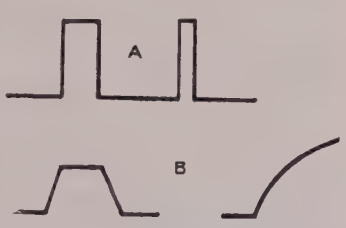

FIG. 79.

The so-called Law of Du Bois Reymond.—In 1848 Du Bois Reymond, as a result of the study of the galvanic excitation of nerve, formulated a law which was for long regarded as a general law of excitation. Since the researches of Waller, Hoorweg and Weiss, it has been known that this so-called law is only a partial statement of what happens under particular conditions, but owing to the important part which it has played in the development of the subject, as well as to the fact that it is still often quoted, we must consider it briefly.

The facts upon which the law was enunciated may be illustrated by the use of the simple liquid potentiometer referred to above (p. 282). If the nerve of a frog's sciatic-gastrocnemius preparation be placed on the electrodes EE, and the cursor C be placed in contact with O, no current will flow. Now let C be *quickly* moved along the groove, say, to 20 cm., so as to keep contact with the fluid and cause a rapidly increasing current to flow through the nerve; the nerve will be stimulated as shown by contraction of the muscle.

Now return the cursor to O and move it away very slowly; it will be found that although it be moved far

beyond the previous distance (say to 50 cm.), no contraction will follow—the slow slope of current-increase is ineffective, whereas the steep gradient stimulated the nerve.

Du Bois Reymond expressed this fact by stating that "*Excitation is a function of the differential coefficient of the current density with respect to time*," or $\frac{dC}{dt}$, that is to say that excitation depends, not on the current density, but on the rate of variation of current density from moment to moment; the more rapid the change the greater the excitatory effect.

This statement is now known, chiefly as a result of the researches of Lapicque, Keith Lucas and others within relatively recent years, to be only partially true. It is true to say that variations of the current, either increase or diminution, are capable of stimulating, but it is not true to say that the process of excitation is limited to the period in which the current is changing. We should now regard it as more correct to say that the current excites during the first moments of its passage, whether variable or constant.

If Du Bois Reymond's law held good, then once the current has been established at a certain value and ceases to vary, it ceases to have any further effect. The simple illustration with plain muscle given on p. 282, since it shows that a certain *duration* of current is also necessary, suffices to show that the law cannot be retained as a working hypothesis, though with the simple apparatus there described it would appear adequate for the striated nerve muscle preparation, which would respond as well with the short stimuli of one-tenth second as with stimuli of several seconds' duration. This is because we cannot, with such an arrangement, get currents of sufficiently short duration; if we could, then, as will appear presently, we could demonstrate that with *any* tissue a minimal current which will excite at a longer duration fails to do so when it is abbreviated beyond a certain limiting value. This limit for the

frog's stomach is of the order of seconds, while for the sciatic nerve it is of the order of thousandths of a second.

These simple considerations serve to illustrate the importance of the time factor in excitation, and to show that "slow" and "rapid" are purely relative terms when different tissues are under consideration. It will be our business in the present chapter to find some method of taking

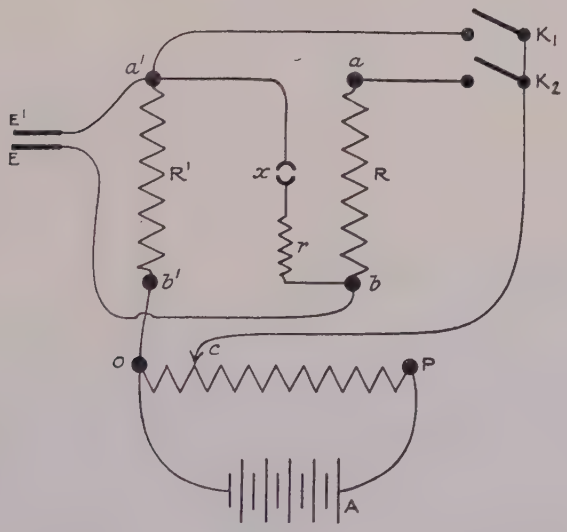

FIG. 80.

into account this time factor, so that we may compare one tissue with another.

Arrangements for Passing Currents of Short Duration. Exciting Circuits.—Currents of known strength and of known short duration can be obtained in a variety of ways. As a starting point it is best to consider currents established and stopped instantaneously, and to consider the gradiented currents later. Apparatus for giving currents of known duration are called *rheotomes*, and of these there are various

types suitable for different durations. In general principle these usually operate by *opening* two circuits at a given time interval, the circuits being as in Fig. 80, which shows the arrangement used by Lapicque.

A is an accumulator, and P a potentiometer from which the required potential is tapped off at *o* and *c*. The resistances R, R′ and *r* are respectively 7,000, 10,000 and 3,000 ohms,[1] E, E′ are the (non-polarisable) electrodes, and *x* is a shunting key. The two keys K_1 and K_2 are the ones which, opening one after the other at the required interval, give a current of that duration through EE′ in the following manner: *x* being open, K_1 and K_2 are both closed, and the current from the potentiometer is short-circuited through K_1. When K_1 is opened the circuit passes from *o* to *b′*, R′, *a′*, E′, E, *b*, R, *a*, K_2 to *c*, thus starting the exciting circuit. When K_2 is opened this circuit is broken and the exciting current is stopped.[2] This arrangement will only give instantaneous establishment and break of a circuit provided that the circuit is free from capacity and self-induction. Coiled resistances or wires must therefore be avoided. Lapicque recommends the use of resistances of graphite, made from lead pencils.

Rheotomes.—We must now discuss the various arrangements by means of which K_1 and K_2 of the circuit just

[1] Large resistances are used in the circuit to avoid dealing with very small potentials on the potentiometer. These particular values are chosen so that R′ = R + E where E = resistance of electrodes + tissue. For muscle this is of the order of 3,000 ohms for electrodes about 1 cm. apart. With these values practically no current flows through EE′ when K_1 and K_2 are closed, but when K_1 is opened the total resistance is R′ + E + R = 20,000 ohms, of which the muscle is, say, 3,000 ohms. The voltage between O and C is thus about seven times as great as would be needed if no resistance were used.

[2] The arrangement just described is for stimulating a muscle; when a nerve is to be stimulated the key *x* is closed; the nerve is thus shunted by the resistance *r* during flow of the exciting current, so that in spite of the much higher resistance of the nerve, it is excited by about the same potential as the muscle, for the resistance between E and E′ is now bridged by 3,000 ohms through which most of the current passes, only a small fraction of it passing through the nerve in parallel with it, which has a resistance of 50,000 to 100,000 ohms.

described may be opened at the requisite short interval of time.

For very short intervals (from 3 σ to 0·20 σ) the only really accurate instrument is the *ballistic rheotome*, introduced by Weiss in 1901 and used, with modifications, by Lapicque. In this instrument the keys K_1 and K_2 are represented by pieces of thin brass wire, which are placed across the trajectory of a bullet fired from a pistol of 6 mm. bore. When the pistol is fired the projectile, travelling at about 26 cm. per σ, cuts through these wires ; first K_1 is severed,

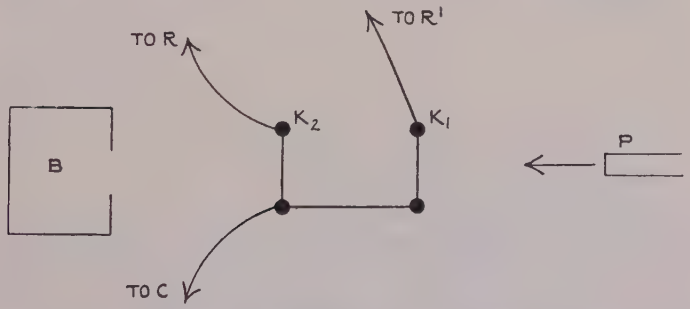

Fig. 81.—Plan of ballistic rheotome. P, the pistol ; trajectory marked with arrow. K_1, K_2, the brass wires severed by flight of bullet. B, iron box to catch bullet. Other letters as in Fig. 80.

which closes the excitatory circuit ; then K_2, placed at the required distance from K_1, is severed, opening the circuit ; if, for example, K_1 and K_2 are 5 cm. apart, the duration of the current will be about 0·2 σ. A diagram of the arrangement is shown in Fig. 81.

This apparatus, in spite of its accuracy, has its drawbacks ; it cannot, for instance, be used for clinical work, since, as Lapicque remarks, " on ne peut guère songer à tirer des coups de pistolet dans un cabinet de consultation."

Pendulums have often been used as a convenient means of opening two circuits, but most of the older instruments had too long a swing to give short intervals with any

accuracy. In 1908 Keith Lucas devised a very short and heavy pendulum (Fig. 82), consisting of a heavy mass B, to which was attached a long rigid arm A to magnify the movement.

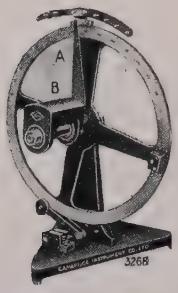

Fig. 82. — Keith Lucas's pendulum.

Two knock-over keys are placed at the required distance on the path of the long arm; one of these keys is fixed to the base, the other is attached to a graduated circle, so that it can be fixed at any desired angle from the first. The apparatus gives a very large range of intervals, from about 0·5 σ to 100 σ, and is thus an extremely useful instrument.

A simplification of this instrument, the spring rheotome, which uses a spring instead of the pendulum, was also introduced by Keith Lucas (Fig. 83), and though less accurate is very useful.

The Chronaximeter of Lapicque [1] has certain advantages for clinical use, since the first key is *closed*, and thus the

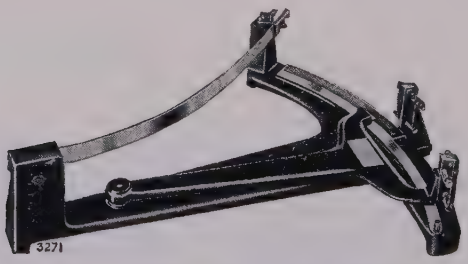

Fig. 83.—Keith Lucas's spring rheotome.

double system of resistances shown in Fig. 80 is not essential. The principle of the apparatus is shown in Fig. 84.

The keys B and A are flat springs which are pressed down and remain down by turning of the eccentric cams B and A. A very rapidly moving arm travelling in the direction of the

[1] Manufactured by Boulitte, of Paris.

arrow first turns down the cam B, thus closing the exciting circuit, and then travels on, and after the required interval,

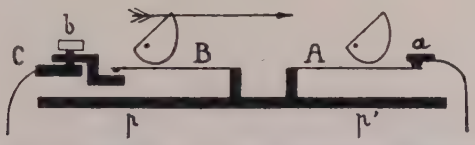

FIG. 84.—Diagram of the keys operated in Lapicque's chronaximeter. (Lapicque, "L'Excitabilité en fonction du temps.")

dependent on the distance of A from B, turns A down and so opens the circuit. The actual arrangement is shown in the diagrams, Figs. 85 and 86.

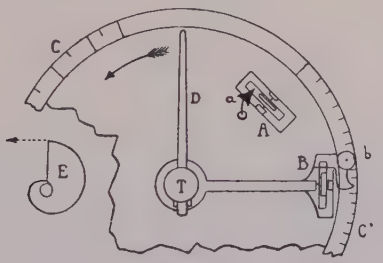

FIG. 85.

The steel needle D is moved very quickly from the zero, in the direction shown by the arrow, by the pull of a weight

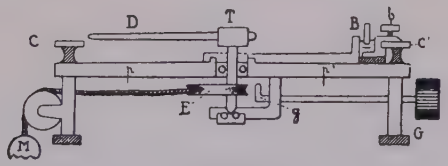

FIG. 86.

M on an eccentric pulley E, released by turning the screw-head G. Contact B can be moved to any position on the arc of the graduated circle; contact A is fixed. Towards

the end of its sweep, the needle D, by reason of the gravity acceleration of M and by the eccentric shape of the pulley E, is moving very rapidly, about 10 metres per second, so that by placing B very near to A, as shown in Fig. 85, a duration of $0{\cdot}25\,\sigma$ can be obtained with accuracy. With the use of a technique for graduation involving the use of condensers, even smaller durations may be obtained [1] with accuracy.

Non-polarisable Electrodes.—The electrodes used for stimulation should of course be non-polarisable, as otherwise the current used would not remain constant. Fig. 87 shows an oscillogram of the current passing through a frog's gastrocnemius at a potential of four volts, using platinum

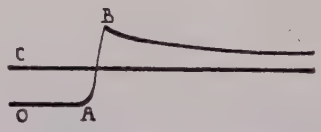

FIG. 87.—Oscillograph tracing. Polarisable electrodes. (After Lapicque.) 1 cm. = about 1/100 second.

electrodes. At A the circuit was closed, and the current rose at once to B, but quickly fell off again; after one or two seconds it had fallen to the level C, about half its original value. This is because the ions present in the tissue fluids move towards the electrode of opposite sign and thus give rise to a *back electromotive force.*

Since the process of excitation itself is probably of the nature of a depolarisation, it is essential in studying it to avoid the complication of a polarisation phenomenon in the electrodes themselves.

With one exception, polarisation always results when a

[1] See Lapicque, "L'Excitabilité en fonction du temps," 1926, p. 332. The method consists essentially in using the current of the duration given by the chronaximeter to charge a condenser, which then on the opening of the second key discharges through the nerve of a nerve-muscle preparation. Since the minimal voltage of the condenser discharge necessary to excite is easily determined and since the rate of charging of a condenser is expressed by a well-known formula, this method provides a simple way of measuring short intervals of time.

current passes from an ordinary metallic conductor to a saline solution, but not when it passes from one saline solution to another. This exception is when the metal dips into a solution of its own ions, and this fact is taken advantage of in the construction of non-polarisable electrodes. In these a metal dips into a solution of its salt; any tendency for polarisation is at once counteracted at the cathode by deposition of the metal on to the electrode from the solution, or at the anode by solution of the metal of the electrode to neutralise the negative charges carried thence by anions.

Three important types of electrode are the following :—

Du Bois Reymond.

$Zn/ZnSO_4/$ isotonic NaCl solution.

Ostwald.

Hg/Hg_2Cl_2 saturated in isotonic NaCl/isotonic NaCl.

d'Arsonval.

Ag/AgCl/ Isotonic NaCl.

In this last type AgCl is formed at and deposited upon the anode and AgCl is decomposed, with deposition of Ag at the cathode. This electrode, though convenient, is far from completely non-polarisable when any but weak currents are used.

The zinc-zinc sulphate electrodes are already familiar to the student and need not be further described. A drawback is the diffusion of the toxic $ZnSO_4$ into the saline solution; this is greatly reduced in Noyons's modification, in which the $ZnSO_4$ is solidified by gelatine or agar. (Fig. 88 (A) .)

Fig. 88 (B) shows a convenient form of calomel electrode, and Fig. 88 (C) shows one of the most useful types, viz. Lapicque's modification of the d'Arsonval electrode. The silver rod is coated with silver chloride by electrolysis,[1] and the electrode tube is made of ruby glass to avoid the action of light. For many purposes it suffices to use silver wires

[1] Of NaCl solution ; 2 volts is used, the silver being the anode.

freshly coated by electrolysis and placed in direct contact with the tissues.

A further point of importance is the distance of the two electrodes apart; this should be at least one centimetre,

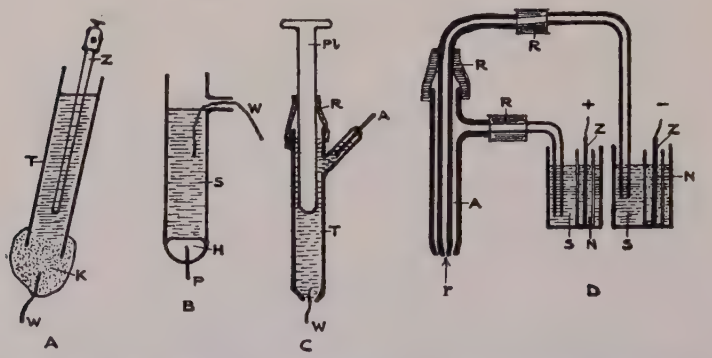

FIG. 88.—(A) Diagram of Noyons's type of non-polarisable electrode. Z = amalgamated zinc rod imbedded in 10 per cent. gelatine containing $ZnSO_4$ and contained in the glass tube, T. K = plug of kaolin made with isotonic NaCl solution. W = thread wick soaked in isotonic NaCl leading to tissue.

(B) Diagram of Ostwald type of N.P.E. P = platinum wire making contact with mercury H. S = isotonic NaCl solution containing solid calomel. W = saline wick lead to tissue.

(C) Diagram of Lapicque's AgCl electrode. A = silver electrode coated with AgCl. Pl = plunger fitting rubber sleeve R, for filling electrode with NaCl solution. W = saline wick lead.

(D) Diagram of Pratt's pore electrode. P = the fine pore which is the stimulating point. The tube is filled with saline solution and connected with the saline solution in the vessel S, in which is a porous N.P.E. cell filled with $ZnSO_4$ solution and having a Zn rod immersed in it. The outer tube, A, is the other electrode and is similarly connected with the other N.P.E. cell. R = rubber connections.

otherwise the chronaxie is liable to be too low, for reasons given by Rushton.

Whatever type of electrode is used, it is essential that the stimulating surface be as small as possible; Hallowell Davis has shown that with large electrode surfaces, abnormally long chronaxies are obtained. A convenient type of electrode is, for this reason, the " pore electrode " designed by

Pratt, shown in Fig. 88 (D). This is a non-polarisable electrode with a very fine orifice (only a few μ in diameter), which is used as the exciting electrode in conjunction with a large indifferent electrode, or as in the figure, with an annular indifferent electrode surrounding it. By its use a single muscle fibre can be stimulated, and the effect observed under the microscope if necessary. If the microscope is not employed, it is necessary to consider that contraction has occurred as soon as there is the smallest visible twitch of the muscle.

Some tissues, for reasons not explained, give irregular chronaxies; the sartorius appears to be an example of this.

The Effect of Galvanic Currents of Short Duration.—By the use of condenser discharges to be described subsequently, it had been inferred that the time factor played an important part in excitation, but by the use of rectangular waves of short duration obtained by the ballistic rheotome, Weiss showed directly that when the duration fell below a certain limit, a minimal effective stimulus could only be obtained by the employment of a higher voltage. Below this limit the shorter the period of flow of current the higher the voltage necessary to excite. He further showed that, if a very short wave, A, was broken into two, B and C (Fig. 89) with a gap between, contrary to the expectation of Du Bois Reymond's law, the voltage necessary to excite was not lowered, but raised. Hence it cannot be the mere variation of the current which excites.

FIG. 89.

Nernst, on theoretical grounds, deduced the formula $i = \frac{K}{\sqrt{t}}$ or the liminal intensity varied inversely as the root of its duration. This could, however, only apply over a limited range, for it is evident that as t becomes infinitely great, i

should become infinitely small, and we shall see that this is not the case.

Weiss's Laws.—When the liminal voltages were plotted against the shortest duration necessary to excite, a curve of the rectangular hyperbola type (Fig. 90) was obtained, one of

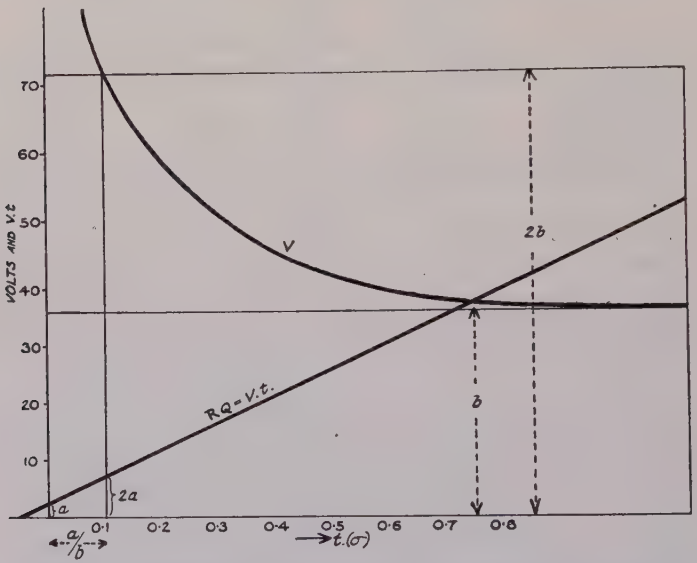

FIG. 90.—To illustrate Weiss's laws and their relation to chronaxie. $a = 3{\cdot}5$. $b = 36$ volts. $\frac{a}{b} = c\ 0{\cdot}1\sigma$ = chronaxie. Abscissæ = time in $0{\cdot}1\ \sigma$. Ordinates figured = volts. Ordinates for RQ = Vt are on same scale. (Modified from Bourguignon. *La chronaxie.*)

the asymptotes of which is a line at the level of the threshold for infinite duration.

If the quantities (Ct) [which when R is constant are $\propto$ Vt] are plotted against times, a straight line is obtained as a first approximation (Fig. 90), though as will be shown later it is not exactly straight. These relations are expressed by Weiss's laws :—

(1) For intensity.

$$i = \frac{V}{R} = \frac{a}{t} + b.$$

(2) For quantity.

$$Q = it = a + bt,$$

where a and b are two constants.

a is the theoretical liminal quantity of electricity for zero duration (Fig. 90); b is an intensity, and is the asymptote of the hyperbola, which in practice is the liminal stimulus at infinite duration, *i.e.*, a duration beyond which time is without further effect.

It follows from these two constants that both intensity and time, or quantity, are concerned in excitation. The ratio a/b was regarded by Weiss as the characteristic of excitability of a tissue, and this has been confirmed by L. and M. Lapicque. This ratio a/b of quantity to intensity is evidently a time.

When the intensity is equal to twice the liminal strength it is evident that the duration of the liminal exciting current is equal to a/b; for since in Weiss's formula $i = a/t + b$, and this $= 2b$,

therefore $$\frac{a}{t} = b\text{; or } t = a/b.$$

Chronaxie

Lapicque calls the liminal value for indefinite duration (galvanic threshold) the *rheobase*, and replaces the ratio a/b by what he has called the *chronaxie* which is indicated by the letter τ.

The chronaxie may be defined as the shortest duration of twice the rheobasic strength which will stimulate, and it is easy to see that it may be derived from the formula of Weiss as follows:

$$i = \frac{a}{t} + b,$$

$$i = b\left(\frac{a}{bt} + 1\right),$$

and replacing a/b by τ this becomes

$$i = b\left(\frac{\tau}{t} + 1\right),$$

i.e., $i = 2b$ when $t = \tau$.

Although there is this approximate relation between chronaxie and Weiss's law, the definition of chronaxie does not depend on the correctness of this law, which, as will be

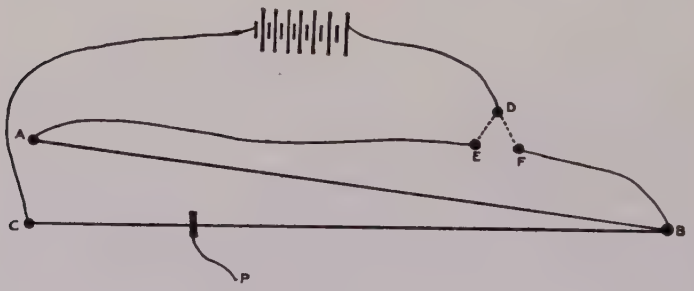

Fig. 91.

shown later, is not exact, but is an empirical constant enabling the characteristics of excitability of tissues to be compared irrespective of the exact relations between duration and intensity. The advantage of taking into account both of these parameters of excitability is that as a matter of practical utility, whereas the rheobase varies considerably with such circumstances as the surface of the electrodes, their distance apart, etc., the chronaxie, provided the electrodes are not less than a centimetre apart, varies only with variations in the excitability of the tissues.

In practice, in order to determine the chronaxie, all that is necessary is to find the galvanic threshold or rheobase, using an ordinary key, double the voltage so employed, and

then, using a suitable rheotome, find the shortest duration of this which will excite.

In order to double the rheobase one of two procedures is adopted. The most obvious way is to double the reading of the potentiometer employed to vary the voltage; the other method does this without calculation by the use of a simple device shown in Fig. 91.

DEF is a 2-way switch, allowing D to connect with *either* E or F. The potentiometer wire consists of two parts, AB and BC, of exactly equal length; BC may be graduated if desired; the potential is tapped off along BC by the cursor P. To commence, the connection DE is made and then the rheobase is found by sliding P along BC; then connection DF is made, which halves the length of the potentiometer wire and thus doubles the voltage drawn from the length CP.

The following table shows some approximate chronaxies for excitable tissues of very varying types :—

Tissue,	Chronaxie (σ).	Approximate Duration of Contraction of Muscles.
		Seconds.
Frog's gastrocnemius (esculenta)	0·3 σ	0·1
,, ,, (temporaria)	0·7 σ	—
Frog's sciatic (esculenta)	0·3 σ	—
Rectus abdominis (esculenta)	0·9 σ	—
Toad's sciatic gastrocnemius	1·3 σ	0·16
Frog vaso-constrictor fibres	2·0 σ	—
Sudo-motor fibres in cat's sciatic	3·0 σ	—
Frog's ventricle	3·5 σ	1·0–2·0
Helix foot	5–25 σ	—
Tortoise heart (testudo græca)	8·2 σ	2–3
Claw muscle of crab	30 σ	5
Frog stomach	30–100 σ	15–20
Mantle of Aplysia	80 σ	—
Spirogyra	8,000 σ	—
Pigment cells, skin of frog	11,000–15,000 σ	—

Significance of Chronaxie

The chronaxie of a tissue is a definite measure of its excitability, since in that time a current of twice the rheobasic strength is able to produce those physico-chemical alterations which determine excitation. The more excitable the tissue is, the more quickly will this current produce the changes which culminate in excitation. It can be shown by comparison of the strength-duration curves of different tissues that what happens in a highly excitable tissue in, say, 3/10,000th of a second, may require, say, 8 seconds for a very inexcitable one.

If we could imagine, for instance, that the tissues possessed an appreciation of time, we could suppose that they measured it, not in the astronomical units of seconds, but in the physiological units of chronaxies. Physiological phenomena could thus be compared in different tissues by expressing data for each tissue in terms of its own chronaxie.

It is evident from consideration of the figures for different chronaxies that there is a relation between chronaxie and speed of action of tissue. The more excitable tissue has, as a rule, a shorter chronaxie, shorter period of action, shorter refractory period, and a quicker rate of propagation than a less excitable one.

(*a*) **Effective Duration.**—When a constant current of rheobasic strength is passed for an indefinitely long time it exerts an excitatory effect only during the first few moments, and then ceases to produce any further effect. This effective period is called the *effective duration* of the flow (*temps utile ; nutzzeit*). Though this period is not determinable with great accuracy, it may be found approximately by ascertaining the longest time which, on reduction, will necessitate a raised liminal voltage. For example, suppose that a frog's sciatic gastrocnemius is excited by passage of a current with an e.m.f. of 0·2 volts applied for 3·0 σ ; if the time is reduced to 2·0 σ, the voltage must be increased ; if it is lengthened

to 4·0 σ the liminal voltage of 0·2 produces no greater effect. The effective time is thus about 3·0 σ.

Experimental determinations of the effective times of some tissues gave the following results (Lapicque) :—

Mantle of Aplysia	800 σ
Adductor of crab's claw . . .	300 σ
Tortoise heart	80 σ
Foot of *Helix*	50 σ
Gastrocnemius of frog . . .	3 σ

It will be seen that these durations are about eight to ten times as great as the chronaxie of the tissues concerned ; in other words, the constant current exerts an effect during 8–10 chronaxies, or, reciprocally, the constant current of abrupt onset must last for at least 8–10 chronaxies before it will stimulate at the rheobasic strength. If the current is of gradual onset, its effect will be abolished if the establishment takes longer than 10 chronaxies to reach rheobasic strength.

(*b*) **Law of Isochronism.**—On examination of the excitabilities of various motor nerves, and of their muscles, Lapicque arrived at the conclusion that under normal conditions the nerve had the same, or nearly the same, chronaxie as the muscle which it supplies. It has for long been known that slowly-contracting muscles are supplied by relatively inexcitable nerves.

(*c*) **Velocity of Propagation.**—Here, again, the relationship between chronaxie and response is suggested, for we find that just as in frog's nerve with a chronaxie of 0·3 σ and a propagation velocity of 30 m.p.sec., *i.e.*, about 1 cm. per chronaxie, so in other nerves the velocity of propagation is of the same order per chronaxie.

(*d*) **Speed of Reaction of Tissue.**—Comparison of the speeds of contraction of different types of muscle shows that the slower contracting tissue has the longer chronaxie ; no generalisation seems to be possible on quantitative lines, however. With nerve, the action current is known to last

longer with nerves supplying slowly contracting muscles, *i.e.*, with nerves of high chronaxie, and there are indications of a rough relationship between the chronaxie and the duration of the ascending phase of the current of action. Lapicque suggests that this ascending phase endures for 3 chronaxies, and though this figure requires confirmation, it is extremely likely that two processes so closely associated as these measures of time relations of excitability and excitatory state respectively will be found to be quantitatively related to one another.

(*e*) **Chronaxie and Size of Nerve Fibres.**—There appears to be a close connection also between the chronaxie and the size of nerve fibres, and the following table, after Kriendler, shows that the smaller fibres are less excitable (longer chronaxie) than the larger :—

—	Chronaxie in 1/1000 μF.	Average diameter in μ of Fibres supplying Muscle.
Semi-membranosus . .	30	17·5
Biceps	40	16
Gastrocnemius . . .	50	13
Sartorius	50	12
Flexor of digits (hind limbs) .	85	9·5

(*f*) **The Summation Interval.**—Keith Lucas has shown that the greatest time interval at which two stimuli of a strength less than the threshold strength by a known percentage may follow one another and yet just succeed in provoking an excitation also varies with the chronaxie. That is to say, the excitatory process subsides most quickly in the tissue which is most excitable.

(*g*) **Electrode Concerned.**—Where the excitability of the tissue is different at the two poles, the chronaxie is dependent on and characteristic of the condition at the cathode, and not elsewhere.

Determination of Chronaxie by means of Condenser Discharges

The passage of an electric current in rectilinear waves of very short duration presents, as we have seen, some technical difficulties, and it is desirable, for the investigation of the phenomena of excitability, to be able to pass waves of short but accurately known duration without having recourse, except as a standard of reference, to the somewhat cumbrous ballistic rheotome. With certain reservations, this want is filled by the use of condensers, which enable waves of short and measurable duration to be obtained. First of all, one must briefly glance at the laws of condenser discharges.

Law of Condenser Discharge.—When a condenser of capacity C (in farads) is connected with a source of potential of V (volts) it soon becomes fully charged, and then holds a quantity of electricity $Q = VC$ (coulombs). If the condenser is now allowed to discharge through a circuit (in which a tissue may be introduced), the discharge takes a certain length of time, which, comparable with the emptying of a water tank, depends upon the quantity of electricity in the condenser, $Q = VC$, and on the resistance R (in ohms) through which, in discharging, it has to pass.

Further, like the emptying of the water tank through a pipe offering a definite resistance, the flow of electricity is not constant throughout the discharge, but starts off at a high value, which falls off and finally ends in a mere dribble. The capacity of the condenser corresponds, in hydrostatic analogy, to the cross-section of a tank of indefinite height; the voltage to the height to which it is filled; the quantity Q to the product of the two. By charging condensers of different capacities to the same potential, or condensers of the same capacity to different potentials, different quantities of electricity are stored.

An important law of the discharge of a condenser is expressed by the equation :

$$i = \frac{V}{R} e^{-\frac{t}{RC}},$$

where i = the intensity of the current of discharge (in ampères) at the moment t.
R = the resistance of the circuit (in ohms).
e = the base of natural logarithms (2·7183 approx. : log. of 10 to base e = 2·302. . .).
t = the time in seconds during which the condenser has been discharging.
C = the capacity of the condenser (in farads).

At the instant when discharge begins (when $t = 0$), then, since $e^{\circ} = 1$, in accordance with Ohm's law $i = \frac{V}{R}$. This rapidly falls off, and although theoretically the discharge is never complete, it can be shown from the above formula that the current falls to $1/n$th of its initial value when $t = RC \log_e n$.[1] If a condenser of 1 microfarad is discharged through a resistance of 10,000 ohms, for instance, the intensity of the discharge after 3 σ is about three-quarters of that immediately on closure of the discharge circuit, after 7 σ about one-half, after 14 σ about one-quarter, and after 20 σ about one-eighth, etc. (Fig. 92, A). One of one-tenth the capacity would have a similar curve of discharge, but falling ten times as rapidly, so that in 3 σ the intensity would be only 0·075, in 7 σ about 0·05, in 14 σ 0·025, etc., of its initial value (Fig. 92, B). If the discharge is allowed to pass

[1] *E.g.*, with a condenser of 1 μ F and R = 10,000 ohms the current is half its initial value when

$$\begin{aligned} t &= RC \log_e 2. \\ &= 10^4 \times 10^{-6} \times 2{\cdot}3 \times \log_{10} 2{\cdot}0. \\ &= 10^{-2} \times 2{\cdot}3 \times 0{\cdot}3. \\ &= {\cdot}0069 \text{ seconds,} \end{aligned}$$

and by a similar calculation it can be shown that in 0·115 secs. the intensity of discharge is reduced to one-millionth of its initial value.

through an excitable tissue, excitation will occur provided the voltage and duration of the discharge are adequate; this excitatory effect will cease, however, before complete discharge has occurred, as soon as it falls to a certain value.

The careful investigations of Lapicque have shown that under appropriate conditions the condenser discharge may be used for determination of chronaxie, the effective duration of the discharge through constant resistance being proportional to the capacity of the condenser. When the

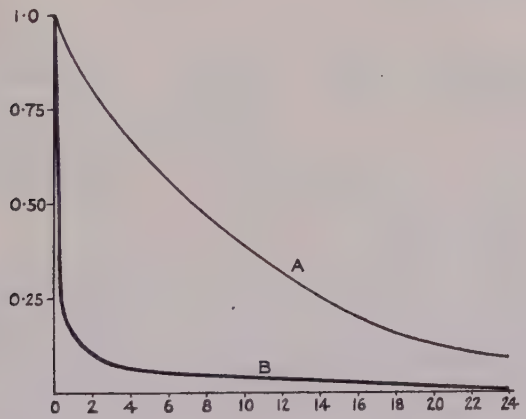

FIG. 92.

resistance and the capacity both vary, the duration of discharge is proportional to the product RC, called the *time constant.*[1] It is much easier to obtain currents of very short duration by the adjustment of condenser capacities than by any of the rheotomes for use with constant currents, and the drawbacks attendant on the use of discharges of rapidly declining strength have been overcome by careful comparison of the results of the two methods, as will be shown later.

Apparatus Employed.—The arrangement used is shown

[1] RC is, in fact, in dimensions a time, for in electromagnetic units capacity $= L^{-1} T^2 \mu^{-1}$ and resistance $= LT^{-1} \mu$; hence $RC = T$.

diagrammatically in Fig. 93. The variable condenser C is charged to the required voltage from the potentiometer P, when the spring key K is pressed down for a second or so. When K is released the charging circuit is broken, and discharge of the condenser occurs through the resistance system and electrodes in contact with the tissue. The condenser actually consists of a number of accurately

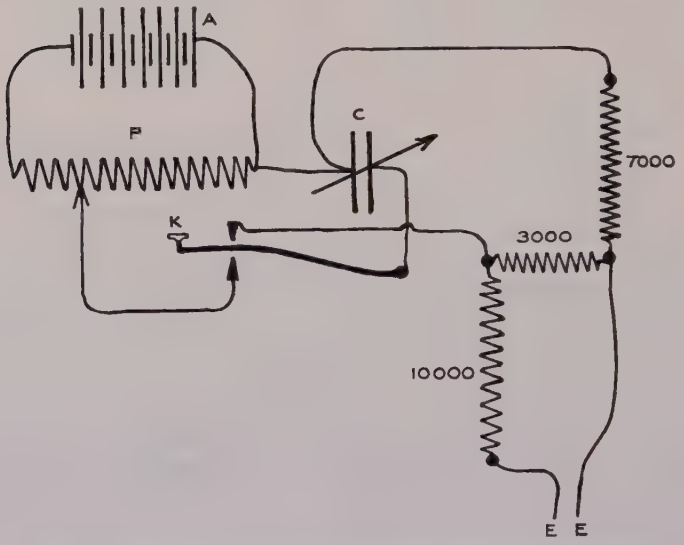

FIG. 93.—Arrangement for chronaxie determination by condensers. (After Lapicque.)

standardised fixed condensers ranging in capacity from $\frac{1}{1000}$ to $\frac{1}{2}$ microfarad, and one or two large ones of approximately 2 microfarads, the last to serve as an indefinitely large capacity for determination of the rheobase.

The accurate capacities are conveniently chosen as follows :—

1 of 0·001 microfarad	1 of 0·04 microfarad
2 ,, 0·002 ,,	1 ,, 0·1 ,,
1 ,, 0·005 ,,	2 ,, 0·2 ,,
1 ,, 0·01 ,,	1 ,, 0·5 ,,
2 ,, 0·02 ,,	

The total capacity of the set is thus 1 microfarad, and any fraction of it down to 0·001 μF can be obtained by the proper combination, in parallel, of suitable capacities.

Method.—First of all the rheobasic strength (potential) is determined, either by direct use of the constant current from the potentiometer, or more usually by the use of an indefinitely large capacity, which will give a duration of discharge greater than the effective duration for the tissue. The necessary voltage is then doubled, and by repeated trial the smallest capacity which will excite on discharge from that potential is determined. The chronaxie proper is always proportional to this capacity when the resistance is constant, or to RC if resistance varies. As explained above, the product RC is the time constant of the condenser, and is given in seconds if R is in ohms and C in farads.

If, for example, $R = 20{,}000$ ohms $= 2 \times 10^4$ ohms
and $C = 0{\cdot}023\ \mu F = 2{\cdot}3 \times 10^{-8}$ F,
then $CR = 0{\cdot}46 \times 10^{-3}$ seconds $= 0{\cdot}46\ \sigma$.

This time constant is, however, not the chronaxie proper, though it varies proportionally to it. By comparisons of time constants determined in this way with chronaxies determined by the ballistic method, L. and M. Lapicque find that the true chronaxie $= RC \times 0{\cdot}37$, the average factor 0·37 determined empirically being called the factor of transformation.

Thus for the gastrocnemius sciatic of the frog the following results were found :—

(*a*) By condenser method.

$$R = 20{,}000,\ C = 4 \times 10^{-8},\ RC = 0{\cdot}8\ \sigma.$$

(*b*) By ballistic method.

$$\tau = 0{\cdot}3\ \sigma$$

$$\text{Factor} = \frac{0{\cdot}3}{0{\cdot}8} = 0{\cdot}375.$$

The factor 0·37 is the average figure found from a large number of observations of this nature, and hence all that is

needed in order to determine the chronaxie by condensers is to find the capacity C (in farads) which will just excite when charged at twice the rheobasic voltage and discharged through a known resistance R (ohms), and the chronaxie in seconds then = 0·37 CR. Since the capacity required can be determined to the nearest 0·001 μF, that gives, with a resistance of 20,000 ohms, a result accurate to an order of 0·00001 second (or 0·01 σ), far greater, in other words, than can be secured by the use of ordinary rheotomes. This is a great advantage when dealing with short chronaxies.

Laws of Excitation by Condenser Discharges. Hoorweg's Laws.—Investigation of the excitation of a tissue by discharges, through constant resistances, of condensers of different capacities shows that with condensers below a certain capacity the liminal voltage increases as the capacity diminishes, while above that capacity the liminal voltage is the same for all capacities. When we remember that the duration of discharge depends on the product RC, it is clear that this relationship of voltage and capacity is similar to the one already studied between voltage and duration of rectilinear waves. Hoorweg, in 1892, expressed these relationships in the form of equations like the following :— [1]

(1) $V = a/C + b$, the law of voltages, comparable with Weiss's law of intensities (see p. 294), subsequently discovered for rectilinear waves ; voltages plotted against capacities for liminal stimulation give a hyperbolic curve similar to that of voltages or (intensities) plotted against duration.

(2) $CV = a + bC$, the law of quantity, exactly comparable with Weiss's law, where t is substituted for C, and, like it, giving a practically straight line when CV is plotted against C.

[1] The lettering of Hoorweg's constants was different from that given here. In all the above equations R is ignored because it is kept constant and therefore C is a function of time.

The constants a and b have similar significance in the two sets of formulæ, and need not be further discussed.

(3) As the capacity increases the energy of the discharge first diminishes and then increases.

The energy of a condenser discharge is $1/2V^2C$, and $E = \frac{1}{2}C(a/C + b)^2 = \frac{1}{2}\left(\frac{a^2}{C} + 2ab + b^2C\right)$; the minimum value for E is when $C = a/b$.

Now a/b, as we have already seen, is identical, or almost identical, with the chronaxie; in other words, the duration of condenser discharge which will effect stimulation with the minimum expenditure of energy is one which coincides with the chronaxie of the tissue.

This fact of a point of minimal energy for stimulation was observed by Waller in 1899, and the following extract from his paper shows the relations of falling voltage, rising quantity and first falling, then rising, energy as the capacity is increased.

Frog's nerve. R = 70,000 ohms. Minimal stimulation.

Capacity C. (microfarads).	Voltage (volts).	Quantity V.C. (microcoulombs).	Energy 0·5 CV^2 ergs.
0·00035	1·44	0·000504	0·00363
0·00085	0·72	0·000612	0·00220
0·002	0·36	0·000720	0·00130
0·007	0·18	0·001260	**0·00113**
0·05	0·09	0·004500	0·00202
10·0	0·045	0·45000	0·10125

Waller gave the name of "characteristic" to the ratio $V/RC \times$ constant for that minimal stimulus at which the energy was also minimal. It is virtually the same as $\frac{\text{rheobase}}{\text{chronaxie}}$, and hence less valuable than chronaxie.

The laws of Hoorweg, like those of Weiss, are only approxi-

mations. This is shown most clearly by exact study of the law of quantities, which according to these laws should give a straight line graph.

L. and M. Lapicque have found, for example, that for any tissue it is only between certain limits that the graph of quantities in either case is a straight line. For short times (or small capacities) the curve is concave towards the abscissa, while for the other extreme it is convex to the abscissa. The departure from the straight line is, however, less with long than with short chronaxie tissues.

Determination of Chronaxies in Man

The problem of the determination of the chronaxies of the human nerves or muscles presents many difficulties; these have been very thoroughly investigated by Bourguignon and his associates, with the result that accurate measures of these values are now available for purposes of investigation or diagnosis.

In the first place, when motor points or nerves are stimulated, the electrode must be very accurately applied to the required point and kept there during the whole measurement of rheobase and of chronaxie. Specially constructed silver-silver chloride electrodes are used for this purpose, with plugs of asbestos soaked in 4 per cent. NaCl. These are applied by hand, and not fixed.

Secondly, it is impossible either to maintain the subject's electrical resistance constant or to determine it with accuracy, and hence a shunted circuit similar to the laboratory one is used, in which the resistance and the variations in the resistance of the subject are negligible. When the resistance does not vary the durations of the condenser discharge will depend only on the capacity. This circuit must, however, enable at least 15 to 20 milliamps to flow with an E.M.F. not exceeding 200 volts. Condensers are used for the excitations of variable duration, the assembly being composed as

described on p. 304, but ranging from 0·01 microfarad to 20 microfarads.

A simplified scheme of Bourguignon's arrangement is shown in Fig. 94.

As shown in the diagram, one branch of the shunt has a resistance of 10,000 ohms, the other being composed of the

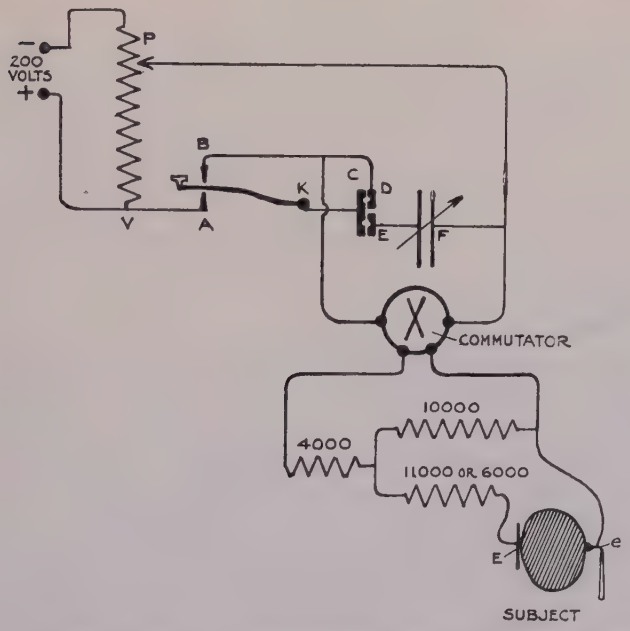

Fig. 94.—Diagram of arrangements used by Bourguignon to determine chronaxie in man.

subject plus 11,000 ohms, or, if this gives too feeble an intensity, 6,000 ohms is substituted.

In series with this shunted circuit is 4,000 ohms. Calculations show that with this arrangement, if the resistance of the subject varies between 2,000 ohms and 50,000 ohms, the resistance of the shunted part of the circuit only varies from 5,650 ohms to 8,590 ohms, or of the whole circuit from

9,650 to 12,590 ohms, *i.e.*, it does not vary by more than 10 per cent. from a mean value of about 11,000 ohms, and this value is accordingly taken as the resistance of the whole circuit through which the condenser discharges. Then, since R = 11,000, it follows from Lapicque's formula,

$$\tau = RC \times 0{\cdot}37$$

$$\text{that} \quad \tau = C\mu \times 4\sigma,$$

where $C\mu$ = capacity in μF. Thus a condenser capacity of 1 μF yields a chronaxie of 4 σ.

In making a determination, the large indifferent electrode E is placed on the sternum, and the small stigmatic electrode *e* applied to the requisite point on the skin; the points C and D are put in contact by the plug, C and E being disconnected. This gives a galvanic current, with which the threshold for the negative pole is found; the current is, however, passed an equal number of times in both directions (the commutator being used) to diminish polarisation. The voltage is read and doubled, and the plug moved from CD and put between CE. The requisite condenser capacity is then found (key on A = charge, key on B = discharge), and the calculation made. During the whole observation the operator holds the electrode *in situ* and attends only to the keys and commutator; an assistant deals with the potentiometer and the condenser adjustments. After completing the chronaxie measurement, the rheobase must be verified before shifting the electrode from its position.

It sometimes happens that the motor point chosen gives a chronaxie which is obviously too high. This is because the area over which a galvanic threshold may be obtained is larger than that responding to condenser discharges, the current from which appears to spread less; both areas have the same centre. It is necessary therefore to find the optimal place when the condenser discharge is being used, and this may be done by first searching for it with a small

capacity condenser discharge, and then proceeding to measure rheobase and chronaxie at that spot, as described above.

This technique has been very carefully controlled by Bourguignon. He has shown, *inter alia* (1) That the intensity of the current traversing the tissues is proportional to the voltage employed. (2) That the chronaxies calculated from different experimental data (*e.g.*, voltage or intensity) agree with one another. (3) The chronaxie of a muscle at its motor point and of its motor nerve are identical (law of isochronism). Certain nerves contain fibres which innervate muscles of different chronaxie. An example of these is seen in the nerve supply to the frontal part of the occipito-frontalis and to the corrugator supercilii, the former of which has a chronaxie double that of the latter, measured at the motor points. When the branch of the facial nerve supplying these muscles is stimulated in the temporal region it is found that the chronaxie required to cause each muscle to contract is the same as that of the muscle itself at its motor point.

	Stimulation of Nerve.				Stimulation of Motor Point.			
	Rheobase.		Chronaxie.		Rheobase.		Chronaxie.	
	Volts.	Milli-amps.	Capa-city (μF.).	$\tau\sigma$.	Volts.	Milli-amps.	Capa-city (μF.).	$\tau\sigma$.
Corrugator supercilii	25	0·8	0·07	0·28	35	1·1	0·06	0·24
Frontalis .	27	0·9	0·13	0·52	36	1·2	0·15	0·60

A similar observation can be made with the supinator longus ($\tau = 0{\cdot}08 - 0{\cdot}16\sigma$) or an extensor ($\tau = 0{\cdot}44 - 0{\cdot}72\sigma$) supplied by the radial nerve.

(4) That the presence of the skin, while altering the rheobase, leaves the chronaxie unaltered is also demonstrated by comparison of indirect and direct excitation in the rabbit, as the following experiment shows :—

Motor Point of Rectus Femoris : Rabbit.

Stimuli to :	Rheobase (volts).	Chronaxie (capacity μF.).	Chronaxie (= RC × 0·37) (R = 10,000). τ
Skin surface . .	34	0·02	0·074 σ
Exposed muscle . .	8	0·02	0·074 σ

(5) Lastly, the technique involving condensers has been checked with the use of the ballistic rheotome, which gave identical results—

Progressive Currents

When the exciting current, instead of being of sudden onset, as with the rectangular wave or condenser discharge, rises on a slope to a particular value, it is found that excitation depends on the *rate* of current rise as well as on the actual intensity finally reached. Hence, for a particular rate of rise or gradient there is a particular liminal value of exciting current and *vice versâ*, the gradient of current increase here representing the time factor in the stimulus. A current that is just able to stimulate at a given gradient will fail to stimulate if the gradient is reduced, and if the slope is made slow enough even a very strong current will fail to excite. For relatively inexcitable tissues, such as plain muscle, a very slow gradient will still excite, whereas for frog's striated muscle a relatively steep gradient is essential.

The subject of current gradients necessary to excite has been investigated by numerous workers, and important contributions were made by Keith Lucas, who devised an ingenious apparatus for giving currents increasing in linear

relation to time. With this apparatus he was able to show that there is a liminal gradient, and slower slopes than this fail to excite. For the sciatic nerve of the toad, for instance, the minimal gradient was found to be such that the current strength which would have been reached if the rate of increase continued for one second was forty-six times that required to stimulate on instantaneous closure. This is a measure of the gradient of the *limiting slope*. For the sciatic nerve of the frog the limiting slope was found to be that of a 63-fold increase per second. The frog's nerve, therefore, has a steeper limiting slope than the toad's, just as it has a shorter chronaxie.

An ingenious experiment of Lapicque's illustrates very clearly the different limiting slopes for the toad's and frog's sciatic nerves. If a condenser is allowed to discharge through a resistance circuit, including a nerve, and having another (uncharged) condenser of equal capacity " shunted " across the electrodes, then the discharge passing through the tissue will rise along a slope to its maximum and then fall again as the second condenser becomes progressively charged and discharged. The rate of rise of the discharge through the nerve may be calculated from the capacities of the condensers and the resistance of the circuit. If the two condensers are chosen of equal capacity and the sciatic nerves of the frog and toad, with their gastrocnemii attached, are both placed on the electrodes, then it is found that with small charges only the frog's muscle will respond when the condensers are of appropriately small capacity (steep gradients), and only the toad's muscle when the capacities are larger (slower gradient).

A question which naturally arises is the following : During what length of time is the current active in provoking the excitatory state when the limiting slope has been reached ? The results of Keith Lucas, von Kries and Lapicque indicate that the full effect is reached in about 30 σ for the frog's sciatic and (Lucas) about 50 to 60 σ for that of the toad. In

the case of the frog's nerve, therefore, the current at the liminal gradient exercises an effect over a period about ten times as long as that of the effective duration of a current of abrupt onset—in other words, the current of liminal linear gradient in order to reach the threshold requires ten times as much electricity as the current of abrupt onset.

With progressive currents, the form of the slope at onset is by no means a matter of indifference ; thus a linear slope is more effective than an exponential one, as Lapicque has shown.

The Law of Isochronism

Direct and Indirect Excitation.—The same chronaxie will be found for a muscle whether it be stimulated directly or *viâ* its nerve, in spite of the fact that the rheobasic strength is much greater for direct than for indirect excitation. The classical view is that the muscle is much less excitable than its motor nerve, and that between the nerve and the muscle there is interposed a material, related to the motor end plate and having special properties, by which the excitation is passed from nerve to muscle. There may be said to have been three views with regard to the nature of the motor end plate. According to one view originated by Rouget, the motor ending, in the frog at all events, is merely an expansion of nervous material, in direct contact with the muscle substance. Another view, based on the work of Kühne, is that the ending consists of a fine ramification of neurofibrillar material, also in direct contact with sarcoplasmic substance. In many animals, such as the lizard, the nerve endings ramify in a nucleated syncytium or end plate which is probably of sarcoplasmic origin, but from this appearance there grew up the third view, that there was a special intermediate material, neither nerve nor muscle, interposed between the two.

It is difficult if not impossible at the present time to say exactly how the idea of the intercalated material of the end

plate arose, but the suggestion may have been due to Kühne that the action of curari, discovered by Claude Bernard, was on an intermediate end plate substance, and not, as Claude Bernard supposed, on the motor nerve substance itself.

This hypothesis of an intermediate or receptive substance, whether demonstrable by histological methods or not, gained some ground as the result of the investigations of Elliott on the myoneural junction in plain muscle, of Langley on the action of nicotine and other drugs on striated muscle, and further from the experiments of Keith Lucas on the excitability of muscle. This material, which was supposed to translate the nervous impulse into an excitation of the muscle, was also believed to be the site of action of curari and other drugs such as nicotine, physostigmine or strychnine, and also to be the seat of fatigue processes leading to loss of response of the muscle after prolonged excitation of its nerve. A familiar class experiment proves that when the nerve is repeatedly stimulated, it is not the main nerve trunk itself which suffers fatigue, and in the same demonstration it is usual to show, by direct excitation of the muscle, that this is not exhausted either; the readiest inference is therefore that some intermediate material is the seat of the fatigue process, and a very small and apparently justifiable leap led to the identification of this hypothetical substance with the morphologically demonstrable granular material of the motor end plate. Once the idea was admitted, it was easy to ignore objections and to furnish additional proofs in support of it.

The investigations of Elliott on the action of adrenaline had furnished very good grounds for the belief that, in plain muscle at all events, there is some intermediary substance, the myoneural junction between nerve and muscle tissue. After section and degeneration of the nerve, the motor action of adrenaline is retained, if not augmented; certainly after removal of the superior cervical ganglion the effect in

causing dilatation of the pupil of minute amounts of adrenaline is greatly increased.

The Receptor Substance.—Langley's exhaustive study of the action of nicotine and curari on striated muscles led him to similar conclusions. When a solution of nicotine is applied to a surviving voluntary muscle, twitching contractions, followed by a prolonged tonus, result. This effect is obtained much more promptly and with much weaker solutions (*e.g.*, 1/1000th as strong) when these are applied to the muscle in the region where the nerve enters it than when applied to other parts of the muscle. After section and degeneration of the motor nerve the effect of nicotine persists. Hence, Langley argued, the nicotine acts on something distal to the nerve endings, since these disappear on degeneration of the motor nerve. Curari abolishes the effect of nicotine, and hence also acts peripherally to the nerve ending. Conversely, nicotine applied in sufficient concentration to a curarised muscle will antagonise its effect and cause a tonic contraction.

The stronger the solution of nicotine applied, the stronger must be the curari solution necessary to abolish its action. But curari does not appreciably antagonise the action of the stronger solutions of nicotine on the non-neural portion of the muscle. From consideration of these and other results Langley concluded that there was a " receptor substance " distal to the motor nerve ending, which had an affinity for, and therefore specifically combined with, certain drugs such as nicotine and curari, which then exerted excitatory or paralysing effects upon it.

It is not difficult to find objections to all of these arguments. One of them, based on chronaxie, will be given shortly. A mechanical objection to some aspects of Langley's nicotine experiments is suggested by Fulton, namely, that in the neural region of the muscle the hypolemma is thin or absent, so that drugs would penetrate more readily at such points : after degeneration has been complete for some time the

local action of nicotine is reduced, and this corresponds with the histological observation that the sarcolemma ultimately grows over the site of the end plate after degeneration has occurred.

A further point which appears to support the view of the receptor substance is the undoubted fact that the excitatory process in passing from the nerve to the muscle suffers a definite " end-plate delay," which in the frog is about 3 to 4 σ. An alternative explanation of this delay is, however, that the rate of conduction is very much slowed in fine nerve fibres, such as those in which the motor fibres terminate.

An argument apparently in favour of the receptor substance was advanced by Keith Lucas in 1907. If there be such an intercalated material it might reasonably be expected to possess an excitability different from that of either muscle or nerve proper, and he claimed that his experiments in 1906, with condenser discharges, and those in 1907, with galvanic currents, supported this view, and showed the presence, especially in the toad's sartorius muscle, of three excitable materials, called α, β and γ, of which the β-substance was believed to be the receptive material, α being muscle and γ nerve. In some cases the presence of all three substances could be shown in the same piece of tissue from the middle of the muscle, the excitation (voltage-duration) curve of which, as illustrated in Fig. 95 A, showed three separately sloped parts. The pelvic end of the muscle, which is nerve-free, only gave the curve of the α substance. After light curarisation (0·01%) the excitability *viâ* the nerve was abolished, but the curve of excitation showed an inflection corresponding with the α and β substances, as in Fig. 95, B. Heavier doses (0·03%) of curari could abolish the β substance response and leave only the smooth curve ascribed to the α substance (Fig. 95 C).

A different explanation of these results is now possible owing to the experiments of Jinnaka and Azuma, Hallowell Davis, Adrian and the Lapicques. Lucas used large fluid

electrodes and, with these, very large and variable chronaxies are obtained, especially when a large surface of tissue is available. Lapicque points out that when the curves of quantities are plotted from Lucas's experiments, quite impossible discontinuities are revealed. Using the pore electrode of Pratt, Jinnaka and Azuma obtained

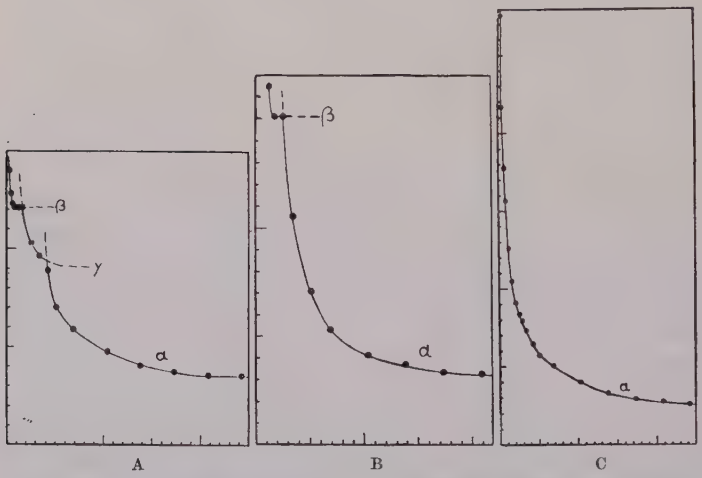

FIG. 95.—A = voltage-duration curve from middle portion of the toad's sartorius, showing the presence of the α, β, and γ types in one experiment. Abscissæ = duration, each division being 1 σ. Ordinates = liminal voltages, each division being 0·1 volt.

B = similar curve after treatment with 0·01 per cent. curari.
C = similar curve after treatment with 0·03 per cent. curari.
(Keith Lucas, *Journ. of Physiology.*)

chronaxie values of 0·3 σ for the single muscle fibre at the pelvic end of the sartorius of the frog, *i.e.*, a value similar to that obtained for both muscle and nerve by the Lapicques, who used fine chloride-coated silver electrodes. Davis confirmed this, and showed (as Adrian showed for the frog's ventricle) that much longer chronaxies are obtained with fluid electrodes. Finally, Fulton, in Lapicque's laboratory, found that, using the Lapicque technique, a perfectly

smooth curve was obtained from the normal frog's sartorius (Fig. 96).[1]

There is thus no evidence for the existence of the β-substance, while at the same time Lapicque's view of the isochronism of the muscle and its motor nerve is strengthened.

The Action of Curari and other Drugs.—Much of the argument for the existence of the receptor substance having turned on the action of drugs, it is now necessary to examine Lapicque's views on this subject, which are exceedingly

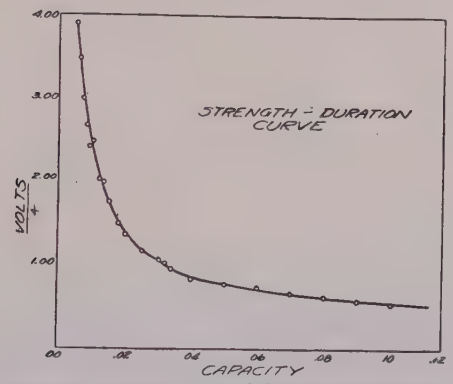

FIG. 96.—Strength-duration curve of the sartorius muscle of the frog. (Fulton, "Muscular Contraction," 1926.)

simple and readily put to the test. Briefly, his thesis is that at all events approximate isochronism of nerve and muscle is a necessary condition for the transmission of the excitatory condition from nerve to muscle. Failure of transmission may be expected to happen when the chronaxie of the muscle differs definitely from that of its nerve, actually when there is a discrepancy of about 100 per cent.

Examination of the action of those drugs which abolish the transmission supports this hypothesis. Curari and

[1] Dr. W. A. H. Rushton has privately communicated to me some results of experiments which appear to me to throw some doubt upon Lapicque's view of the above phenomena. Pending the expression of Professor Lapicque's opinion upon these observations, however, I understand that Dr. Rushton would prefer me not to refer to them.

sparteine act by increasing the chronaxie of the muscle, that of the nerve remaining unaltered : conduction fails when the chronaxie of the frog's muscle reaches about 0·6 σ. Strychnine (0·25%) on the other hand reduces the chronaxie of nerve without changing that of muscle, and indirect excitation fails when the chronaxie of the nerve has been reduced to 0·15 σ. The following table shows these effects :—

—	Effect on Chronaxie of	
	Muscle.	Nerve.
Curari, sparteine . . .	+	0
Strychnine	0	—
Veratrine, nicotine and physostigmine	—	0
Scopolamine	+	—
Hypertonic saline . . .	+	+

In any case as soon as the chronaxie of either tissue is double that of the other conduction fails ; by the further action of the drug much greater discrepancies in chronaxie are reached ; with large doses of curari the chronaxie of the muscle may be as much as 60 times the normal. When the course of action of curari was studied by frequent determinations of the chronaxie during the onset of poisoning it was found that the rheobase for *indirect* stimulation steadily increased, but the chronaxie remained unaltered until excitability disappeared altogether ; on *direct* excitation of the muscle the same increase of rheobase was seen, and here also the chronaxie was unchanged or almost so, until practically the time at which conduction from the nerve was abolished, when it sprang up to about twice its original value. This is interpreted as showing that in so-called direct excitation it is, or may be, the intramuscular nerves which are excited under ordinary conditions, so long as the excita-

bility is transmissible to the muscle, but after the chronaxie discrepancy has become too great, then the muscle is excited direct.

By the use of progressively rising currents the Lapicques have been able to obtain a current of such slow rise as will be capable of exciting the slow-chronaxie curarised muscle, but not the quickly responding normal muscle.

Capacity (μF).	Duration of Establishment in 100th second.	Liminal Voltage.	
		Before Curari.	After Curari.
0	0 (Rheobase)	0·60	0·70
0·3	0·6 ,,	0·62	0·70
1·0	2·0 ,,	0·90	0·81
3·0	6·0 ,,	1·60	1·10

This shows that the slow muscle chronaxie which appears after the action of curari is actually produced by the poison and is not an independent muscular chronaxie unmasked by the disappearance of the nervous component. These facts provide a simple explanation of the action of these drugs on the muscle-nerve preparation and also of the antagonism which some of them show. Thus the effect of strychnine is antagonised by veratrine, physostigmine or nicotine, and that of curare by physostigmine or nicotine. This explains some at least of the results of Langley.

Watts disagrees with the actual experimental results of Lapicque and adheres to Keith Lucas' view, as he claims that although the chronaxie of the muscle may be altered, this is not a *sine qua non* for failure of conduction.

The Influence of Fatigue.—The failure of transmission which happens when a nerve-muscle preparation is stimulated indirectly is also explicable on the lines of a chronaxie increase of the muscle similar to that effected by curari. The chronaxie of the muscle steadily increases, while the

nerve, apart from an increase of the rheobase, shows no change; when the chronaxie of the muscle reaches a value double that of the nerve the conduction is abolished, *i.e.*, fatigue is apparently complete. By direct excitation, however, the muscle can be made to execute further contractions, but with a progressive increase in its chronaxie. On allowing the muscle to rest its chronaxie falls again, and when it reaches a value about double the nerve chronaxie, its indirect excitability begins to reappear. The following results of an experiment illustrate this:—

—	Muscle.		Nerve (with shunt).	
	Rheobase.	Chronaxie.	Rheobase.	Chronaxie.
Before fatigue .	1·4	1·0	0·6	1·0
After fatigue by 200 stimuli . .	1·2	3·5	—	—
After ten minutes' rest	1·5	1·8	—	—
After further ten minutes' rest .	1·5	1·5	5·0	1·0
After further ten minutes' rest .	1·4	1·0	2·0	1·0

This effect of fatigue cannot apparently be attributed merely to accumulation of acid, for Bourguignon, Turpin and Guillamin, and also de Waele, find that decrease of the H-ion concentration of the body generally in man by hyperventilation, or of the animal by injection of alkalis, increases the chronaxie, while addition of acids reduces it. Adrenaline is found to reduce the chronaxie of the muscle and accelerate recovery from fatigue.

The Effect of Section of the Nerve.—For some time after section of the motor nerve the chronaxie of nerve and muscle remains unaltered; this period may be up to a week or ten days in the sciatic-gastrocnemius of the frog, and at the end of this time the nerve has ceased to function. The chronaxie

of the muscle remains normal for a while longer and then steadily increases, so that at the end of three months the muscle chronaxie is up to five times its normal value. In warm-blooded animals and in man the change takes place much more rapidly and has been extensively studied by Bourguignon. The following experiment is an illustration :—

—	Rheobase (volts).	Chronaxie. σ
(Dog) normal chronaxie of inferior gemellus (" jumeau externe ")	—	0·40
Do. fifteen days after division of sciatic, the observations being made on the exposed muscle .	31·2	16·3 (bipolar method.
	10·0	15·9 (unipolar method).

The muscle after degeneration of its nerve gives a slow contraction and is frequently of such high chronaxie as to be inexcitable to the brief faradic shock (reaction of degeneration) ; changes in the chronaxie are, however, detectable long before this stage is reached.

In man the increase of chronaxie is less rapid if the muscle is caused to contract, or in those disorders where there are fibrillary or chorea contractions. As Bourguignon points out, change of chronaxie is related not to any particular disease, but to particular changes of muscular function. His monograph should be consulted for further particulars of normal and abnormal chronaxies in man.

Chronaxie of Human Muscles

In the course of his studies of chronaxie on man, Bourguignon made the important observation, already referred to on p. 311, that different muscles (with their nerves) have different chronaxies, and further, that in the same segment of a limb, muscles of similar function have the same chro-

naxie. Thus when the chronaxie of flexor and extensor muscles is compared it is found that the flexors have a chronaxie only half as great as the extensors. This agrees with the relative duration of the contractions, which Baird and Fulton find to be about 30 σ and 40 σ respectively.

Of muscles with similar functions, those of a distal segment of a limb have a chronaxie about twice as great as those of the proximal segment. Extensor muscles may be divided into two groups :—

(*a*) Extensors proper with characteristic extensor values.

(*b*) Extensors synergic for flexion, with the same chronaxie as flexors.

The following table illustrates these points :—

Muscles.	Chronaxie.	Functions.
Deltoid Biceps Brachialis anticus Supinator longus	0·08 to 0·16 σ	Flexion of forearm and arm.
Internal head of triceps	0·08 to 0·16 σ	+ 1 synergic (int. head of triceps).
External head of triceps Long head of triceps	0·16 to 0·32 σ	Extension of forearm.
Pronators, superficial and deep flexors, palmar muscles and brachio-radialis	0·2 to 0·36 σ	Flexion of hand and fingers and pronation.
Supinator brevis, all extensors and abductor longus pollicis	0·44 to 0·72 σ	Extension of hand and fingers and supination.

Other Aspects of Chronaxie

The Heart.—The chronaxies of the sinus, auricles and ventricles are believed to be identical, while the chronaxie of the bundle of His is stated to be about three times that of the remainder of the myocardium. Fredericq proposes to explain the $a - c$ and P — R intervals in the cycle as due to

the fact that the velocity of conduction in the His tissue is, as indicated by its chronaxie, less than in the other parts, and not, as Lewis found, about three times greater. According to Fredericq, stretching of the myocardium decreases the chronaxie. During vagal stimulation M. L. Lapicque and Veil showed that the chronaxie of the ventricle is reduced, and Field and Brücke found that this applies also to the heart, driven electrically at a constant rate and thus not arrested by vagal stimulation. Cinchonidine and quinidine were shown by M. Lapicque and Fredericq to have little action on the sciatic-gastrocnemius, but to increase the chronaxie of the ventricular muscle and other slowly contracting muscles. It will be remembered that Lewis found that vagal stimulation reduces the refractory period of the mammalian auricle, and that quinidine lowers the rate of conduction in the heart muscle and lengthens the refractory period of the auricle.

Drugs which accelerate the heart rate, *e.g.*, atropine, adrenaline or caffeine, have the opposite action, and lengthen the chronaxie.

Latent Addition.—A stimulus of a duration which is short compared with the chronaxie, say, one-tenth or one-hundredth of it, may be too weak to excite a tissue when applied once, or when repeated only a few times, or at long intervals, but if the same stimulus is repeated many times and at sufficiently short intervals a response may follow. This phenomenon of the summation of inadequate stimuli is called latent addition or summation of excitation.

In order to explain it, we may assume that a single stimulus produces a state of excitation, but that this is inadequate to provoke a response from the tissue. This state of excitation may be supposed to arise and then fade away. If a second stimulation is applied before the excited condition has subsided too far, the excitatory state due to the second stimulation is added to that remaining over from the first, and that due to the third to that remaining over from the

second, and so on, until finally the degree of excitation is sufficient to provoke a response.

Under such conditions, the liminal voltage necessary to excite is affected by the following variables :—

(1) The duration of the stimuli (provided all are shorter than the chronaxie).

(2) The number of stimuli given.

(3) The interval between successive stimuli (usually considerably greater than one chronaxie).

By fixing two of these variables in an experiment we can

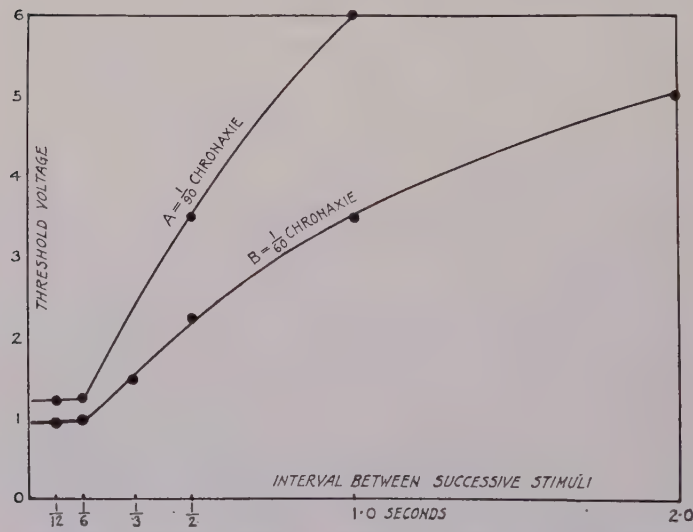

FIG. 97.—Relation between frequency of stimuli and threshold strength in an experiment by the Chauchards (*Ann. de Physiol.*, 1925, 1, 6). Columella muscle of Helix, with chronaxie of 0·04 sec. Resistance of muscle 2,000 ohms. Stimulation by 48 repeated condenser discharges at intervals shown. Curve A, with condenser capacity $= 0{\cdot}6 \times 10^{-6}$ F. ($= \frac{1}{60}$ of chronaxie); curve B with $0{\cdot}9 \times 10^{-6}$ F. ($= \frac{1}{60}$ chronaxie).

find the influence of the remaining one on the liminal voltage to excite, and express the results in the form of a graph. It is easier to carry out experiments on tissues with long

chronaxies because of the ease with which stimuli much shorter than the chronaxie can be obtained. This has been done by the Chauchards, and the results theoretically studied by Lapicque.

When the interval between stimuli is the variable chosen to study, and the results are plotted on a graph, this has the form shown in Fig. 97.

At rapid rates of repetition the threshold is low, and remains constant. Lapicque suggests that this is because beyond a certain limit of frequency of stimulation the refractory period of the tissue or some similar property enters into the phenomenon, and prevents the second stimulation from producing an effect, because of a refractory phase following the first stimulation which has not yet died away.

At the other limit the stimuli are so slow that the state of excitation has completely died away from the first stimulation before the second is applied. At this, or a lower frequency, the threshold strength again becomes constant, and represents the threshold for a single brief stimulation. Between these upper and lower limits of frequency, however, the curve shows an exponential relationship between the threshold stimulus and the interval between successive stimuli. If we suppose that at the application of the second stimulus the excitation due to the first stimulation has fallen to $\frac{1}{q}$ of its original value, calling this original value 1, then the sum of excitation after the nth stimulus will be represented by the geometrical progression $1 + \frac{1}{q} + \frac{1^2}{q} + \ldots + \frac{1^{n-1}}{q}$

This is a convergent series, that is to say, its sum tends towards a finite value which will be $= 1 + \frac{1}{q - 1.}$

It can be seen by simple arithmetical trial that, if this is so, the number of terms necessary to approximate to the

limiting value of the sum of the series is smaller the smaller the fraction $\frac{1}{q}$ becomes.

What is observed as a result of repetitive stimulation agrees with the above argument : if the threshold of excitation is not reached with a certain limited number of stimuli it will never be reached ; again, the more frequent the stimuli are, that is, the greater the value of $\frac{1}{q}$ when each successive stimulation falls, the greater is the number of stimuli which can summate to produce excitation. For example, in experiments by Chauchard for frequencies of the successive values of 12, 6, 3, 2, 1 and 0·5 per second, it was found that the number of stimuli necessary to reach the threshold of stimulation were respectively 48, 36, 18, 10, 6 and 2–3. The law, between limits, set by the " refractory " effect on the one hand, and the threshold for unique stimulation on the other hand, can be deduced mathematically from the argument given above, and experimental results agree sufficiently well to lead one to suppose that the argument is justifiable.

Repetitive Excitation

Repetitive excitation is met with in other examples besides those in which the tissues themselves exhibit the phenomenon of latent addition. We know, for instance, that many visceral responses do not ensue on the application of a single stimulus to the nerves concerned, but will readily do so on repetitive excitation. The reason for this probably lies in the effector tissue rather than in the nerve. One explanation for it is that repetition of the excitation appears to be able to some extent to overcome failure of response attributable to lack of isochronism between the nerve and the tissue. If we assume that the excitation of a tissue is due to the current of action of the nerve, we may perhaps

bring this phenomenon into line with the phenomenon of latent addition, by supposing that the action current is inadequate to stimulate that tissue when only applied once, or a few times, but if sufficiently repeated succeeds in evoking a response. The vagus, the chorda tympani, the nerves of the sympathetic system are nerves which must be repeatedly stimulated before any physiological effects can be obtained from the organs which they supply.

Central Nervous Phenomena

An application of the views of Lapicque to the problems of the activities of the central nervous system brings out many points that offer suggestions for lines of further inquiry. If we assume that the synaptic relations between contiguous neurones are comparable with those between a muscle and its nerve at the motor nerve-ending, then we can see a possible explanation of some of the phenomena characteristic of the central nervous system. For instance, isochronism or heterochronism between neurones might respectively determine the passage or blockage of an impulse, or the phenomenon of facilitation and latent addition, induction, and even the reciprocal action in reflexes, though it would be difficult to explain many other phenomena without invoking special properties for the nerve cells.

An important distinction is made by the Lapicques between the usual chronaxie of a nerve dissected out of the body and one which remains in connection with the central nervous system. The former is called the *constitutional chronaxie*, the latter the *chronaxie of subordination*. They find that the chronaxie of subordination is much shorter than the constitutional chronaxie. Destruction of the spinal cord, section of the motor nerve, or division of the posterior nerve roots alters the chronaxie to the constitutional level. They consider that the chronaxie of subordination is maintained by reflex influences which arise chiefly in the muscle

itself, as a consequence of its tension. Thus, when the foot of a frog is flexed, the chronaxie of the intact motor fibres to the gastrocnemius muscle, which is thereby stretched, is reduced ; if the nerve is divided the stretching of the muscle does not affect the chronaxie of the nerve.

It is at present not possible to propose any theoretical explanation of this reflex control of chronaxie, but the outcome of the discovery, if the facts are fully substantiated, cannot fail to have far-reaching consequences in connection with central nervous phenomena.

Physical Theories of Excitation

By excitation we mean that local alteration in the tissue which constitutes the necessary condition for starting the propagated disturbance. What is the nature of this change ? Most theories have regarded it as being related to a polarisation phenomenon, though the evidence is purely circumstantial.

Nernst's Theory.—Attracted by the remarkable fact that high-voltage alternating currents of high frequency, such as those generated in the Tesla coil, could be passed through the human body without any physiological consequences beyond those produced by their heating effect, Nernst put forward the hypothesis that electrical excitation depended on an electrolytic concentration of ions at membranes impermeable to them, within the tissue. The passage of a current would concentrate anions at the membrane near the positive pole and cations at the membrane near the negative pole. The very fact that an area of excitation becomes galvanometrically negative may, though with considerable reserve, be regarded as an indication that positive ions have accumulated inside a membrane there, and thus have attracted negative ones to its outer surface. If the current alternated with sufficient rapidity, the requisite concentration of ions at either membrane could not be reached. In a later paper he extended his conclusion so as to embrace the effects of constant currents. His mathematical investigations led him to the formula $i/\sqrt{n} = \text{constant}$, which related the least intensity (i) of an alternating current capable of stimulating to its frequency (n), and to the formula $i/\sqrt{t} = \text{constant}$, for relation between threshold current (i) and its duration (t).

These formulæ, however, disregarded the fact that time beyond the utilisation time is negligible, and did not account for the failure of linear progressive currents below a certain gradient to excite. A further fault lay in his mathematical assumption that the two membranes were separated by an infinite distance. It has already been observed that Nernst's formula only fits the experimental facts over a limited range of observations.

Rushton has shown that the relations, in nerve, between threshold and intrapolar length accords with Nernst's fundamental hypothesis. He further shows that when the electrodes are placed *across* the nerve stimuli are ineffective, the threshold varying inversely as the cosine of the angle between the current and the nerve, and this also accords with the general hypothesis of Nernst.

Hill's Theory.—In 1910, Hill mathematically reinvestigated the question in an attempt to find a formula that was free from the defects of the one derived by Nernst. Assuming that excitation is due to a banking-up of ions, it is clear that as it proceeds the concentration of ions caused by the current must be opposed to an increasing extent by the influence of diffusion, the rate of which for a given concentration of ions at one membrane will depend on the gradient, *i.e.*, on the distance from one membrane where the ion is increased to the other where it is diminished. The closer the two membranes are together the greater will be this dissipation by diffusion. By introducing a constant to represent the distance of the two membranes apart, Hill modified the Nernst equation so as more nearly to satisfy the experimental data with regard to excitation by constant currents of variable duration. With regard to the inefficacy of slowly rising currents, Hill assumed that although under such conditions the usual concentration of ions results, the slow rate of its increase fails to set up a propagated disturbance, which he assumes is initiated by a certain rate of breakdown or synthesis of some compound with which the ion was combined. This phenomenon would interfere very little with the simpler case of the relation of current strength to duration at shorter time. For this simpler case Hill's equation is

$$i = \frac{\lambda}{1 - \mu\theta^t},$$

where i = smallest current which will excite.

t = the duration.

λ, μ and θ are constants of uncertain theoretical significance.

λ is the smallest current which will excite, however long it is passed. It is readily determined ; μ and θ are more complex. θ refers to the position at which the changes of ionic concentration are being considered. Where these changes are in the immediate neighbourhood of one of the membranes it = 0·81. In practice

this constant can be calculated from current-duration experiments.

θ is the constant on which depends the shape of the excitation curve, and hence determinations of θ (or more usually of log θ) indicate the excitability of the tissue, the values of log θ being greater, the greater the excitability of the tissue, as the following data of K. Lucas show :—

Tissue.	Effective Duration of Threshold Current.	Log θ.	Chronaxie (Lapicque) for Comparison.
Nerve to gastrocnemius .	0·003 sec.	0·03	0·3 σ
Sartorius muscle . .	0·02 ,,	0·07	Variable
Frog ventricle . . .	2·00 ,,	0·0005	3·5 σ

Nernst's theory, as modified by Hill, suggests an explanation of many of the facts of excitability, such as the greater efficacy of linear as compared with logarithmic progressive currents, limiting slopes, summation of stimuli, etc. The effect of closing and opening stimuli is also explained as follows :—

When the current is closed there will be inside the membrane next to the *cathode* an accumulation of cations (*e.g.*, H′, K′, N*a*′, C*a*″), and next to the *anode* an accumulation of anions (*e.g.*, OH′, C*l*′, PO_4'''). There is thus caused a gradient of positive ions in the tissue from cathode to anode and one of negative ions in the reverse direction, *i.e.*, a polarisation, and it is supposed to be the relative accumulation of positive ions at this membrane which determines excitation, perhaps because, as Hill assumes, it determines a union of these ions with some constituent to form a compound :

$$\text{kations} + \text{unstable substance} \longrightarrow \text{compound},$$

at the anode this compound is broken down :

$$\text{anions} + \text{compound} \longrightarrow \text{unstable substance} + \begin{cases}\text{kation} \\ \text{anion.}\end{cases}$$

When the current is broken the condition is reversed, the excess of compound formed at the cathode breaks down, liberating the ions again, while at the anode the compound is formed again and excitation occurs there. The hypothesis would serve equally well if it were assumed that decomposition instead of formation of the compound were the fundamental change in excitation.

Another hypothesis might be substituted for this, viz., that excitation is essentially a polarisation, but that with passage of the currents there is also an alteration in the permeability of the membranes, so that, *e.g.*, cations slowly penetrate the membrane

and escape from the cells in the neighbourhood of the cathode, or anions in the neighbourhood of the anode, or both. If this were the case, we should expect that opening of the current, by reversing the direction of the stream of ions, would cause excitation at the anode ; also a current rising too slowly would be ineffective because the leakage of ions would keep pace with their migration to either membrane.

The study of the electrical conductivity of tissues lends some support to this ionic leakage hypothesis. Tissues have considerably less resistance to rapidly alternating than to direct currents. When a direct current is passed through a tissue the resistance, at first low, rapidly rises to a high value, *i.e.*, a polarisation such as is postulated to occur at the membranes seems probable. But the resistance does not become so high that the tissue fails to pass any current at all—*i.e.*, a leakage of ions is suggested.

We could not expect the resistance ever to become infinite for another reason, however, namely, because we should always have some conducting fluids on the surface of the tissue, or in its interstices. A more satisfactory proof that the membranes do leak is that the passage of a continued current through a short length of nerve will produce a continued *extrapolar* electrotonic current ; this could not be produced by the interstitial fluids, nor by completely impermeable tissue membranes. (Rushton.) [1]

Lapicque's Theory.—Lapicque, like Hill, has attempted to find an expression in which the inherent defects of Nernst's theory were avoided. He assumed first that excitation follows when the local concentration of an ion resulted in a particular *ratio* of concentrations between the membrane and the interior of the cell, and further that if this ratio is not established in a certain time it never will be reached.

Lapicque constructed an ingenious hydrodynamical model to illustrate his hypothesis that at the polarised membrane the ions are not arrested, but retarded ; thus negatively charged ions are driven from the cathode against the outer surface of the membrane, but pass through at a certain rate. Owing to the accumulation of anions at the outside surface, cations are attracted to the inner surface, and, by intensifying the electric field, progressively accelerate the transit of anions through the membranes. If, then, excitation depends on the attainment of a particular ratio of ionic concentrations between two places, this will be reached more quickly when the current commences suddenly than if it gradually rises in strength.

He regards the core-conductor model as of value in interpreting the effects of polarisation phenomena on the excitability of nerve ; the polarisation process, travelling over the core, may well be supposed to give rise to a molecular commotion which may be

[1] Private communication.

propagated along its surface; in fact, with a mercury core, a definite mechanical wave may be seen to pass along the surface of the mercury if the current is of sufficiently sudden onset.

After an exhaustive examination of the various mathematical formulæ, Lapicque has at length found one which fits most of the observed facts very well. This was derived from considerations of the energy involved in stimulation. With currents of short duration, excitation develops, as in Nernst's hypothesis, parallel with the polarisation, but after the lapse of a certain time, θ, the antagonistic process (*e.g.*, accelerated permeation, or leakage) intervenes to a steadily increasing extent. The magnitude of θ is the factor which determines the differences in the time factor in excitation, and excitation is found to follow the formula

$$i = a\sqrt{\frac{t + \theta + \sqrt{(t-\theta)^2 + 0{\cdot}16\,\theta^2}}{2t}},$$

where a = the threshold for infinite duration, t = the duration and i = the intensity of the exciting current of duration t. How well this formula fits the facts for very diverse tissues is shown by the table below from Lapicque.

Tissue.	a =	θ =	t =	i obs.	i calc.
Spirogyra .	1	32	1 sec.	5·8	5·75
			5 secs.	2·6	2·58
			10 ,,	1·9	1·84
			15 ,,	1·45	1·50
			30 ,,	1·1	1·11
			100 ,,	1·0	1·001
Helix . .	3·1	0·09	0·014 sec.	8·2	7·93
			0·021 ,,	6·4	6·57
			0·036 ,,	5·0	5·05
			0·057 ,,	4·0	4·06
			0·086 ,,	3·5	3·42
			∞	3·1	3·1
Sciatic-gastrocnemius	60	0·0022	0·33 σ	175	157
			0·67 σ	115	112
			1·0 σ	92	92
			1·5 σ	76	76
			2·0 σ	68	68
			2·5 σ	64	62
			3·0 σ	61	62
			∞	60	60

Comparison of this formula with the chronaxie reveals the interesting fact that θ is equal to about four times the chronaxie.

Lassalle's Theory.—More recently Lassalle has reviewed the position, starting from two hypotheses: (1) That the degree of excitation is proportional to the amount of electrical energy supplied in the stimulus; and (2) that the state of excitation, instead of augmenting indefinitely with the duration of the stimuli, rises to a maximum and then declines. He derives theoretically the expression:

$$i = \frac{a}{\sqrt{2}}\sqrt{\frac{T}{t} + \frac{t}{T}}$$

for stimuli of duration shorter than T, which is the effective duration; a is the rheobasic strength. For durations greater than T, the law becomes $i = a$. He finds, further, that $T = 7{\cdot}87\ \tau$, which agrees well with observed facts.

For another mathematical treatment, on different lines, consult the paper by de Almeida.

Lillie's Theory.—A very complete study of the conditions of excitation and propagation of excitatory waves has been made from the physico-chemical standpoint by Lillie, to whose writings the reader is referred for full details. Briefly, Lillie concludes that the phenomena of excitation are dependent upon variations in the composition, permeability or other properties of surface films deposited at phase boundaries.

As an illustration of the type of phenomenon he has extensively studied the behaviour of the film of passive iron which is formed when iron is placed in strong nitric acid (Sp. gr., 1·42). If an iron wire thus treated be subsequently placed in weaker nitric acid (sp. gr., 1·2) it remains, unlike ordinary iron, unaffected, unless activated in some way. Activation may be brought about by electrical, chemical or mechanical means. For example, if the wire is touched by a piece of zinc, it becomes activated at that spot, and turns black and effervesces; moreover, the activation thus initiated spreads as a wave of definite velocity over the whole surface of the wire, lasts for a few seconds, and disappears, the iron becoming passive again. But now the

freshly passivated iron is resistant to re-activation for some minute or so (refractory period), after which the sensitivity to activation returns again. Mechanical stimulation, summation, electrotonus, polar stimulation on electrical activation, etc., can all be imitated on this fascinating model, as can also some of the facts of the time-relations of electric currents, such as the relative inefficacy of slowly rising currents to activate as compared with currents of abrupt onset.

It is agreed that passivity of iron is due to the presence of a film of some oxidation product, and that activation consists in the removal, by electrochemical or mechanical means, of this film ; once this has been effected at a given spot, electrochemical action is set up between the film (cathode) and the active iron (anode) now laid bare, so that the process of activation spreads over the whole surface, with a velocity which depends chiefly on the temperature. As has been explained in connection with other theories of excitation, the existence of electrically polarised membranes in tissues is extremely probable, and it is by no means outside the bounds of possibility that the application of the cathode of an electrical circuit may induce excitation by effecting an alteration of the state of polarisation, and that this change, once started, may spread away from the site of its initiation in consequence of the electrical disturbance (action potential) thus established. It should be noted that, according to this view, it is the resting tissue which is polarised, and excitation consists in a local *depolarisation* at the membrane surfaces.

Nature of the Membranes.—Although the experimental facts of excitability are such that it seems reasonable to hope that an appeal to the properties of membranes may ultimately lead to a satisfactory explanation of the phenomena, we have as yet few indications as to the nature or even the situation of the membranes on which so much of theory has been erected. As in other instances, a layer one molecule thick would suffice theoretically. All cells, it is true, abound with membranes and inter-phase surfaces at which such monomolecular films might be formed, but

it must be admitted that there seems to be little evidence that any specific histologically demonstrable structures either are or are not responsible. Experiments by Rushton with multipolar electrodes have shown that, in order to obtain threshold stimulation of nerve, the values of the potentials at various points along it must be so adjusted that the current leaving through the medullary sheath at the excited point always remains the same. This suggests that the membrane is near to the medullary sheath and forms a cylinder co-axial with the nerve. Histological structures that would correspond with this would be the axilemma or neurilemma, but since we would expect the individual fibrils also to be excitable, a monomolecular bounding membrane at the surface of each fibril would appear more probable.

Ebbecke's Views.—The views so far examined all regard the tissue as an electrolytic polarisation cell, but Ebbecke considers that it is important to take also into account the fact that the polarisable structures also have a definite electrical capacity, *e.g.*, that of the nerve fibre is about 0·01μF, while the intensities used to excite are usually below the polarisation maximum. If we regard the tissue as a condenser, then the excitation of a tissue by a condenser discharge may be regarded as a discharge of one condenser into another. Then if the total capacity of the system exciting condenser + tissue = C_s, and C_a = capacity of exciting condenser and Ct = capacity of tissue, and the threshold quantity $Q = VCs$, then the voltage necessary for different values of Ca will be given by the equation :—

$$V = Q \Big/ Cs = \frac{Q}{Ca} + \frac{Q}{Ct}, \quad i.e., VCa = Q\left(1 + \frac{Ca}{Ct}\right).$$

Calculations based on this formula show that such a system reproduces very plausibly the conditions of electrical excitation of tissues. Thus the effective duration of threshold stimulus is the charging time for Ct, the chronaxie is the time for half-charge ; when the rheobase is doubled the energy minimum is reached, and also the capacity of the exciting condenser is equal to the capacity of the tissue. Curves plotted from such a model for voltage-capacity give results exactly like those of a tissue, and so do curves for quantity and time. In fact, it is clear that Ebbecke's formula amounts to practically the same as that for Hoorweg's law, with different and more significant constants.

In actual tissues, Ebbecke points out in another paper, as had already been done by Lapicque, the conditions are such as could be imitated by a condenser with a high resistance shunt circuit, the latter being constituted in the tissue by permeability of membranes and by various interstitial fluids and inert structures. The more leaky the condenser the greater will its capacity appear to be, *i.e.*, the longer must the current flow in order to charge it

(large chronaxie).[1] It is perhaps for this reason that the dying or degenerated muscle has a higher chronaxie, since the permeability of its membranes is believed to be increased. Also addition of calcium, which renders membranes generally less permeable, reduces the chronaxie of muscle. He further draws attention to the fact that when a "constant" current is passed through an excitable tissue it is, by reason of the polarisation phenomenon, in reality converted into a rapidly dwindling shock as in charging a condenser, a fact which complicates the theoretical aspect of all excitation observations.

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[1] Dr. Rushton has pointed out to me that this argument is fallacious, and that the chronaxie is *smaller* when the resistance shunted across the condenser is smaller, because, although the condenser does not charge so fast, its final charge is, in proportion, still less.

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CHAPTER XI

THE MECHANISM OF POSTURAL REFLEXES AND THE FUNCTIONS OF THE LABYRINTH

All the higher animals normally exhibit characteristic attitudes in repose or action, by which they are oriented with reference to gravity or other forces. When such a pose is disturbed, it is resumed as soon as conditions allow, and to any circumstance which tends to displace the animal from its posture there is offered a resistance which prevents the displacement, or which restores or tends to restore the animal to its former correct attitude.

Static postures are often maintained for long periods of time without fatigue. The state is one of tonic contraction of the muscles concerned, and the assumption of any posture is the result of a delicate condition of balance in the muscular system, which is effected through the agency of the central nervous system. In the maintenance or recovery of posture many separate types of reflex converge to produce a common result, and these reflexes arise from such sense organs as the labyrinth, the eyes, and the muscles themselves.

The removal of the cerebral hemispheres has but little effect on normal posture, or on the righting reactions by which that posture is resumed after having been interfered with. It is clear, therefore, that these reactions are true reflexes, and it would be quite wrong to imagine that the animal knows that its posture is being upset, resents the interference, and voluntarily acts in such a manner as to counteract the offence. In a fully conscious animal, of course,

voluntary resistance may be added to the reflex reaction, though this is not necessarily so.

Even when the higher parts of the nervous system are removed, leaving only the spinal cord, some amount of reflex muscular tonus persists, but this is slight and bears no resemblance to normal posture. If the medulla is left *in situ*, the section being made through it just anterior to the entry of the eighth nerve, the animal passes into the condition known as *decerebrate rigidity*, which is due to an exaggerated state of tonus of those muscles (extensors) which are employed in the posture of standing. Such a decerebrated animal can, in fact, maintain a standing posture. It differs from the normal animal not only inasmuch as the standing posture exhibits great exaggeration of the action of the anti-gravity muscles, but further in the fact that when upset it makes no movements which would tend to replace it in its former standing pose. It was shown by Sherrington that the tonus distribution in the decerebrate preparation is not entirely proprioceptive, but depends, for instance, on the position of the head.

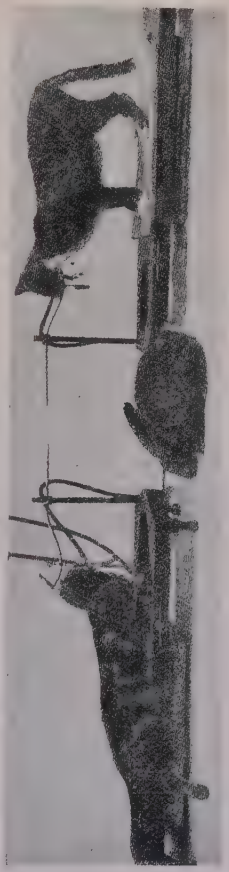

FIG. 98.—(*a*) Decapitated cat, no postural reflexes. (*b*) Decerebrate cat, showing decerebrate rigidity or exaggerated reflex standing. (*c*) Thalamus rabbit, pose normal. (From Magnus, "Körperstellung.")

When the mid-brain is left as well, the attitude of standing

is indistinguishable from that of the normal animal, and, moreover, the animal will now of its own accord return to the standing posture when displaced from it. Most of the postural reflexes of the " thalamus " animal (*i.e.*, with intact thalamus and mid-brain) are practically identical with those of the normal animal. (The advantage of leaving the thalamus is that temperature regulation is retained.) This fact, and the knowledge which we derive from our own experience, teach us that the individual components of sensation upon which posture is based and adjusted to meet varying needs but rarely obtrude into the field of consciousness, and can, therefore, only be studied by objective methods. Such objective studies, based on the examination of the various reflexes which fuse to give the picture of normal postures, have been made by a number of workers, conspicuously by those of the Utrecht school, under the leadership of the late Professor R. Magnus, and the present brief account is largely drawn from his book and other writings on the subject by his collaborators. These studies have shown, to translate his own words freely, " that many different sense organs are involved, that very different centres integrate their stimuli, that the muscles are concerned in many different combinations in the production of the reactions, and that a whole series of diverse reflex groups are balanced together."

The postural reactions of the body when at rest are called *static reflexes*, and fall into two groups : the *stance reflexes* or reflexes of *pose*, in which the body, or the part under consideration, remains in a definite attitude, and *righting reflexes*, by means of which the pose is restored after disturbance. The former, so to speak, " keep you right way up when you are " while the latter " put you right way up when you are not."

The postural reactions of the body when in movement are called *stato-kinetic reflexes*, and are produced in a definitely predictable manner by the movement itself, which in turn is largely guided by them under normal conditions of life.

We will now review some of the important groups of postural reflexes, which, following Magnus's classification, fall into the following heads :—

I. Static Reflexes

(i.) *Local Static Reactions*, *e.g.*, confined to one limb.
(ii.) *Segmental Static Reactions*, involving one segment, *e.g.*, both hind limbs, both fore limbs, or the neck.
(iii.) *General Static Reactions*, involving more than one segment. These may be again divided into :—

A. *Reflex Stance.*

(1) Influence of the Head on Stance.
  (*a*) Tonic Neck Reflexes on Limbs.
  (*b*) Tonic Labyrinth Reflexes.
    (*α*) On the Limbs.
    (*β*) On Neck and Trunk.
  (*c*) Collaboration of Neck and Labyrinth Reflexes.
  (*d*) Indirect Influence of Labyrinth on Limbs through Action on Neck Reflexes.
(2) Indirect Influences on Posture.

B. *Compensatory Pose of Eyes.*

(1) Tonic Labyrinth Reflexes on Eyes.
(2) Tonic Neck Reflexes on Eyes.
(3) Collaboration of Tonic Labyrinth and Neck Reflexes on Eyes.
(4) Collaboration of Compensatory Eye Poses.

C. *Righting Reflexes.*

(1) Labyrinth Reflexes on the Head.
(2) Body Posture on the Head.
(3) Neck Postural Reflexes.
(4) Body Postural Reflexes on the Body itself.
(5) Visual Postural Reflexes.

II. Stato-kinetic Reflexes

A. *Reactions to Rotation.*

(1) Head Reactions.
(2) Eye Reactions.
(3) Reactions on Body.

B. *Reactions to Progressive Movement.*

(1) Reactions of Head.
(2) Reactions of Extremities.

C. *Reactions to Partial Body Movements.*

We must now consider these various aspects of reflex posture.

I. Static Reflexes

(i.) Local Static Reactions

One of the most remarkable facts in connection with the maintenance of stance is that a limb which at one time is freely moveable, at another time is converted into a rigid pillar capable of supporting the weight of the body. For this purpose the anatomical arrangements of the joints and ligaments do not suffice ; the formation of the rigid support is a local reflex, called by Magnus the *positive supporting reaction*, while its resolution, enabling the limb to be used for other purposes, is also a local reflex, called the *negative supporting reaction*. These reflexes receive their explanation from the researches of Sherrington and Liddell, and of Magnus and his collaborators, Rademaker, Schoen and Blake Pritchard.

The afferent stimuli for the positive supporting reflex are in part proprioceptive stimuli evoked by dorsiflexion of the distal parts of the limbs, by which some of the flexor muscles are stretched, and in part are due to exteroceptive stimuli from the skin, *e.g.*, contact of pad of foot with the ground. A direct consequence of the stretching is that these flexor

muscles themselves are reflexly contracted (myotatic reflexes of Liddell and Sherrington); a second result is that there is reflex contraction of flexors and extensors, whether stretched or not, largely by reason of a reciprocal innervation effect, the antagonistic muscles thus acting synergically in fixation, at the proper angle, of the joints concerned. In this complex local reaction very different reflexes co-operate, reinforce, and if necessary replace each other, so that standing under very different positions of the joints is possible.

In the negative supporting reaction, the active stimulus is proprioceptive, due to volar or plantar flexion which unlocks the series of positive reflexes and loosens the limb for action.

(ii.) Segmental Static Reactions

A good example of these is the crossed extension reflex, when, as a result of a powerful stimulus, a flexor reflex is obtained, say, in the front left leg, the increased extension of the right leg enables this to carry the weight formerly carried by both limbs. Again, if a man stands on his right foot and leans, or is pushed, to the left, the resulting reflex extension of the left leg prevents his falling.

(iii.) General Static Reactions

A. Reflex Stance.—(1) *Influence of the Position of the Head.*—Experiments on the effect of the position of the head on the tonic pose of the body are carried out on decerebrate animals, in order to eliminate the righting reactions, which would otherwise complicate the effect. The distribution of tonus of the muscle groups is modified as a result of stimuli from the neck when the head is moved with reference to the trunk, and also, by the operation of labyrinthine reactions, when the head (with the body) is moved in space. These two effects can be separated by proper technique.

(*a*) *Tonic Neck Reflexes.*—These are obtained in a pure form in experiments on animals which have been decerebrated and deprived of both labyrinths, and are also seen in certain clinical cases. When the head is moved into various relationships to the trunk, the extensor tonus of the decerebrate preparation shows modifications which persist so long as the altered relation of head to trunk is kept up. These alterations of pose chiefly affect the limbs, and are due to reflexes which originate in the neck proprioceptors, owing to altered tensions on the neck muscles when the head is moved. It is found that in the alterations of tonus which constitute these responses the muscles of the right and left sides or of the fore and hind limbs behave in opposite ways.

Fig. 99.—Hydrocephalic infant. Turning of head to left gives increased tonus of left arm, due to tonic neck reflex. (Magnus, "Körperstellung.")

When the head is turned so as to rotate on the atlanto-epistropheal joint, the extensor tonus of the fore and hind limbs is increased on the side towards which the nose is turned, and diminished on the other side (Fig. 99). It is the turning of the atlas on the axis which evokes this response.

When the head is inclined sideways by bending the neck, so that, say, the left ear approaches the left shoulder, the extensor tonus of the limbs is increased on that side, while on the right side there is a diminution. This reaction to bending of the neck obviously serves the purpose of keeping

the centre of gravity of the body over the feet when the head is moved to one side.

Raising the head, by which the neck is bent backwards, causes increased tonus of the fore limbs, with, in most animals except the rabbit, reduction of tone of the hind limbs. This results in a posture such as is well seen in a normal cat craning the neck upwards to look on a table. When the head is bent downwards, the tonus distribution is in the opposite direction, as in a normal cat peering over the edge of a wall or eating food.

The latent period for these neck reactions varies from one-third of a second to one second, and the centre which is responsible is located in the upper cervical spinal cord. These reflexes are abolished by section of the posterior roots of the cervical nerves.

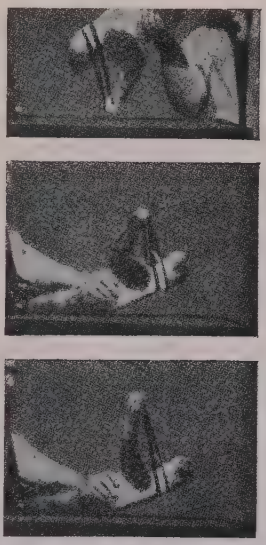

FIG. 100.—Tonic labyrinth reflex on limbs. Decerebrate cat, with neck fixed in plaster and a rubber band passing from fore-paw to neck, to oppose extensor tonus. (*a*) Mouth cleft inclined down, and tonus slight. (*b*) Three seconds after turning animal on back with mouth cleft directed obliquely upwards. No alteration of tonus yet. (*c*) Thirteen seconds after turning over; the tonus is now maximal, and the elastic band is stretched. (From a kinematograph film. Magnus, "Körperstellung.")

(*b*) *Tonic Labyrinth Reflexes.*—These reflexes are best studied on decerebrate cats with intact labyrinths, and which are fixed to a holder in such a manner that the head cannot be moved relatively to the trunk; if necessary, the neck may be embedded in plaster of Paris for complete elimination of tonic reflexes from the neck. When the whole preparation is moved to a different spatial orienta-

tion, the resulting poses may be fairly assumed to be due to the action of afferent impressions from the labyrinths. This is further shown by the fact that deprivation of both labyrinths leads to complete disappearance of the reactions to altered orientation.

When the orientation of the animal is changed, there is, as in the case of the neck reflexes, an alteration in the tonus of the preparation, which persists as long as the evoking position is maintained.

The most conspicuous effect is that on the limbs, all four of which react similarly. Maximal extensor limb tonus is seen when the animal is placed on its back, so that the mouth cleft is horizontal or inclined upwards at 45° to the horizontal; when the animal is moved around a transverse axis through 180°, so that the mouth cleft is again horizontal or inclined downwards at 45°, the extensor tonus falls to a minimum (Fig. 100).[1] At intermediate positions the tonus is of intermediate intensity. Similar results are seen when the animal is rotated around the axis of the vertebral column. If we start with the animal lying on its side, there is an intermediate amount of tonus. Turning it on to its back gives the maximum, and on to its belly the minimum, amount of extensor tonus. If the animal is moved in such a way that the relation of the head to the horizontal plane is unchanged, there is no effect on the state of tonus. Tonic effects of the labyrinth on the neck can also be demonstrated.

The centre concerned in the labyrinth reflexes is placed in the medulla below the entrance of the eighth nerve. The latent period is long, usually over ten seconds. The tonic neck and labyrinth reflexes show an algebraic summation in the normal animal, in which they both have full play. It is also found that the labyrinth exerts an indirect action by

[1] Administration of picrotoxin reverses the tonus of the decerebrate preparation, which then becomes mainly flexor. It is found that the flexor tonus is at a maximum when the animal is placed in the prone position, *i.e.*, when normal extensor tonus would be minimal.

giving rise first to tonus changes in the neck, which produce neck movements, and hence, by the operation of the neck reflexes, exert an effect on the limbs. The two effects of the labyrinth on the body are algebraically summated.

(2) *Indirect Influences on Posture.*—It follows from what has been said that posture is in the normal animal largely influenced by impressions received from the outer world through the senses of sight and hearing. These lead to voluntary movements of the head and, by the operation of the reflexes so far described, to an automatic modification of the pose. To take the cat as an example, we have already referred on p. 346 to certain characteristic postures related to neck reflexes, and a little consideration will show that the pose will be further reinforced by the action of the labyrinth reflexes.

Suppose, again, that a cat hears a mouse moving on its right. The head is turned to the right; this alters the centre of gravity, but, as a result of the tonic neck reflex, there is an increase of tonus in the muscles on the right side of the body, which preserves the balance by throwing the weight on the right limbs. Now, because the left limbs are less loaded, they will be the first to move if the animal springs to its prey, so that the cat will automatically move in the right direction.

B. **Compensatory Pose of the Eyes.**—The compensatory pose exhibited by the eyes under certain conditions is really a special case of stance reflexes. In man and other animals with frontal vision it is of much less importance than in such animals as the rabbit or guinea-pig, or the dogfish, which have lateral vision. Like other muscles, the tonus of the eye muscles may be modified as a result of reflexes arising either from the labyrinth or from the neck. The outcome is in either instance approximately the same; briefly, the eyes take up a position which is such that their visual fields suffer as little change as possible.

(1) *Tonic Labyrinth Reflexes on the Eyes.*—Experiments are

carried out in the manner already described for studying labyrinth reflexes on the limbs and trunk muscles.

In these reflexes the recti externus and internus take no part, and, as with the rest of the body musculature, no effect is produced by way of the labyrinth on any of the eye muscles by movements which do not alter the relation of the head to the horizontal plane. The responses are to rotation of the head (and body) about the occipito-nasal axis (" vertical " response) and rotation around the bi-temporal axis (" rotatory " response). In the performance of these movements there is always a delicately adjusted reciprocal action of antagonistic muscles in such a manner that the eyes continue, as it were, to look at the same object in spite of the movements of the animal. Thus if the animal in the prone position is tilted so that the head moves downwards, the eyes will move upwards, and *vice versâ*.

(2) *Tonic Neck Reflexes on the Eyes.*—In these reactions, which are obtained after destruction of the labyrinth, when the head is moved with relation to the body, *all* the eye muscles are brought into play, and compensatory eye poses are obtained in response to alteration of the head with reference to any plane, including the horizontal. The effects are similar to those obtained with the labyrinth reflexes.

Under normal conditions the labyrinth and the neck reflexes are both present and are summated algebraically, so that, while neither reflex alone is enough to compensate fully for head movements, the addition of the two does so. The exception to this is when the movement of the head is in the horizontal plane, in which case, as already stated, there is no labyrinthine reflex set in operation.

C. **Righting Reflexes.**—The reactions by which an animal keeps itself right side up and recovers its normal posture after it has been disturbed, are seen best in animals deprived of the cerebral hemispheres (thalamus animals), so that voluntary movements are eliminated. An animal with intact nervous system takes up by means of these reactions a

normal stance, which is the basis for other voluntary or reflex movements. Righting reflexes are present in a highly developed form at birth, and are carried out by co-operation of five different groups of reflexes. When, by suitable procedure all of these are eliminated, we have the "*zero condition*," in which no righting reflexes are shown. In rabbits and guinea-pigs this is achieved by destruction of both labyrinths, and holding the animals free in the air; cats, dogs and monkeys, which use the eyes for righting reactions must also be blindfolded if the cerebrum is intact. With the

FIG. 101.—Response of thalamus rabbit to rotation of pelvis when held free in air. The anterior part of the body retains the normal position owing to co-operation of labyrinth, and neck-righting reflexes. View with stereoscope. (Magnus, "Körperstellung.")

zero condition we can compare the effect of introducing the responsible reflexes one by one.

(1) *Labyrinthine Righting Reflexes and Head Pose.*—As a result of these reflexes, the head tends to retain its normal position in space. In carrying out experiments on these reflexes it is necessary when using dogs or cats to exclude visual stimuli by blindfolding, because visual sensations powerfully augment the labyrinthine ones, but this is not so with rabbits and guinea-pigs, which are, therefore, better suited for such experiments, as the reactions in them are the same whether the eyes are available or not.

When a suitably prepared animal is held up in the air by gripping the pelvis (to avoid contact stimuli which would

be introduced if the animal were allowed to lie on the ground), it is seen that the head tends to remain in the normal position when the pelvis is moved to the most various positions. This is accomplished by appropriate twisting or bending of the

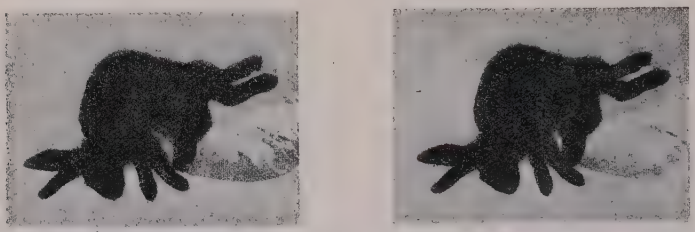

FIG. 102.—Thalamus rabbit after deprivation of labyrinths, for comparison with preceding figure. Stereoscopic. (Magnus, "Körperstellung.")

vertebral column (Fig. 101). After extirpation of both labyrinths or section of the eighth nerves the reflexes are abolished, and the head is allowed to drop by the action of gravity into an abnormal position (Fig. 102).

FIG. 103.—Thalamus rabbit without labyrinths laid on right side. The head, and then the anterior half of the body, assumes the normal pose owing to asymmetric contact stimuli. Stereoscopic. (Magnus, "Körperstellung.")

(2) *Body Posture on the Head.* If a rabbit deprived of both labyrinths be laid on the ground, the head again tends to assume the normal pose. This is due to the operation of asymmetric stimuli arising from contact of the lower side with the ground. Thus, if the animal is laid on its right side, contact stimuli will arise from that side, but not from the

left, and these lead to orienting reactions of the head (Fig. 103). If the stimuli are made symmetrical, as by placing the animal in the lateral position and laying a board on its upper side, then the head orientation vanishes (Fig. 104).

Owing to these two reflexes from labyrinth and from contact stimuli, which tend to keep the head in its normal position, there results a twisting of the neck, and this, in its turn, leads to the production of

(3) *Neck Reflexes.*—These operate in much the same way as the tonic neck reflexes (p. 346), and the result of the combination of all these reflexes is that first the front and

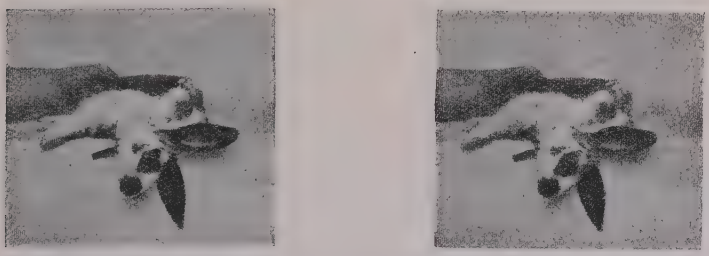

FIG. 104.—The same rabbit as last figure laid on side, but with weighted board placed on upper side; no righting reaction. Stereoscopic. (Magnus, "Körperstellung.")

then the hind limbs follow the posture taken by the head, so that ultimately the whole animal comes to resume its normal pose. For instance, suppose an animal is laid on its left side. In consequence of labyrinthine and contact reactions, the head is turned towards the left shoulder (*i.e.*, remains horizontal); this leads to increase of tonus of the left fore limb, which rights the fore part of the body, then to increase of tonus in the left hind limb, by which the hind part of the body is also righted.

(4) *Reflexes from the Trunk upon the Body itself.*—The effect of asymmetric contact stimuli such as arise when an animal is laid in the lateral position affects not only the head, but also the body itself, so that if the head is fixed in the lateral

position the body may be righted even though this entails opposition to the neck and labyrinth reflexes. In the normal animal there is thus a double reflex mechanism, which is summated to produce the normal effect.

FIG. 105.—Dog sixteen days after double labyrinthectomy, the pelvis held in the air in horizontal dorsal position; righting of head and thorax. Stereoscopic. (Magnus, "Körperstellung.")

(5) *Visual Righting Reflexes.*—These reactions can be studied in cats and dogs with intact cerebral cortex after bilateral labyrinthectomy. A few days after removal of the

FIG. 106.—The same dog held in the same manner, but with the eyes covered; no righting reaction. Stereoscopic. (Magnus, "Körperstellung.")

labyrinths the animals can orient the head quite well if allowed to use the eyes, but when these are covered the animals revert to the disoriented position of the head, just as the labyrinthectomised rabbit does (Figs. 105 and 106).

Centres for Righting Reflexes.—Visual righting reflexes are

cortical. It has been shown by Rademaker, using the method of progressive section at various levels, that the other centres responsible for the righting reflexes are situated in the mid-brain and pons. The neck-righting reflexes are located in the pons, the remainder are in the ventral portion of the mid-brain at about the level of the third nerve nucleus, and probably in the large-celled portion of the red nucleus from which the rubrospinal tract arises. That the red nucleus and rubrospinal tract are chiefly concerned, he has shown by various experiments. Destruction of the pyramidal tract, substantia nigra, or cerebellum leaves the righting reactions unchanged. Mesial sagittal section of the mid-brain cause disappearance of righting reflexes if Forel's decussation has been divided, not otherwise.

II. Stato-kinetic Reflexes

Unlike the righting reflexes, these are produced by the actual movement, and not by any abnormal orientation which it may have produced. In so far as they arise from the labyrinth, they may be said to be due not to movement in itself, but to a change of movement, *i.e.*, to an acceleration, positive or negative. These may be regarded as either angular accelerations, *i.e.*, movements of rotation, or as progressive accelerations when the movement is in a straight line.

A. Reactions to Rotation.—(1) *Reactions of the Head.*—When an animal is rotated, say, to his right, the eyes tend to remain fixed on a given object, and hence the head is steadily moved to the left as far as possible, and then sharply to the right, this movement being repeated, so that a *head nystagmus* results, with the rapid component in the same direction as that of the rotation. After the rotation has been stopped the nystagmus is in the opposite direction, *i.e.*, in the case supposed, with the rapid component to the left.

Turning of the head and head nystagmus are also seen as a result of rotation in other planes than the horizontal. All these phenomena may be explained as due to the effects of the rotation on the semicircular canals.

(2) *Eye Reactions.*—The reactions of the eyes to rotation are similar to those of the head ; that is to say, the eyes tend to remain fixed on the same position as long as possible, and are then brought quickly in the opposite direction to the original position with relation to the head (eye nystagmus). The effect on the eyes is naturally best seen when the head is fixed with relation to the body.

When the head instead of the whole body is moved, the response of the eyes to the movement of rotation so produced leads to their moving in a direction towards that in which they finally become fixed by the static reflexes already considered.

(3) *Turning Reactions on the Trunk.*—Such reactions are not very conspicuous, but are seen in some instances, *e.g.*, in the compensatory movements of the trunk in the lumbar region which we experience when riding in an automobile which takes a sharp bend.

B. **Reactions to Progression Movements.**—(1) *On the Head.*—These are best seen in the dog when placed on a holder in the normal position. When moved suddenly upwards, the head is moved ventrally and the fore-paws flexed ; when the movement ceases, the head moves dorsally, and the fore-paws are extended. On movement downwards the opposite effects are produced.

(2) *On the Extremities.*—When the animal in the normal position is moved upwards, the effect on the fore-legs is as described above. When held hanging head downwards, the reaction to sudden downward movement is that the fore-limbs are moved in an oral direction, as in preparing the animal to take up its weight on falling to the ground. Small differences of reaction are in seen the different species of animals.

C. **Reaction to Movements of Parts of Body.**—When a part of the body is moved, as is often the case as a result of a reflex or voluntary movement, reflex adjustment of pose is often necessitated, and the responses described above are variously brought into play in such adjustments. Thus, for example, if the left hind-toe of a standing dog is pinched, we get a flexor reflex of the left hind-leg, so that the weight of the body has to be borne on three legs, and an extensor reflex of the right hind-leg is one of the adjustments associated with this. When a dog has an ulcerated foot, that leg is held in a position of flexion, and the movements of locomotion are arranged in accordance with the modified support which the body now has. This is all reflexly arranged, and depends on the constant arrival, at the centres concerned, of stimuli from the affected foot. If these stimuli are eliminated, as can be done by applying cocaine to the foot, normal pose and locomotion are at once resumed.

In general we can say that by means of the stato-kinetic reflexes the body is brought into its normal stance, which pose is maintained by the operation of the various static reflexes. It will have been noticed that the head plays an important part in deciding most of these responses. The head contains the organs of special sense, particularly the distance receptors for sight and sound, so that the head tends to be voluntarily directed towards stimuli which affect these sense organs. The proper orientation of the remainder of the body which ensues is effected by the action of the various reflexes which have been described.

Functions of the Labyrinth

(1) **Effects of Unilateral Labyrinth Extirpation.** — The labyrinth on one side may be removed by operative procedure, or its function temporarily suspended by injection of cocaine. The results differ considerably in different species of animals, and are seen in their most developed and elaborate

form in the rabbit. Some of the effects are only transient, others permanent. The results of the studies by the Utrecht school of physiologists, under Magnus, have been to confirm and greatly to extend the findings of the older workers.

In the rabbit unilateral labyrinthectomy leads to skew deviation of the eyes, nystagmus, rotation and lateral flexion of the head towards the side of the lesion, spiral rotation of the neck and trunk, also towards the side of the lesion, diminution of tone of the limbs of the same side and increase in those of the opposite side, and frequently rolling movements, in which the animal turns over and over.

The deviation of the eyes consists in a ventral movement of the eye on the side of the lesion and a dorsal movement on the opposite side. In cats and dogs the deviation is lateral. The nystagmus is transitory, with the slow component towards the affected side and the quick one back to the mid-line. The deviation of the eyes becomes maximal if the head is passively rotated to the normal side, but the nystagmus stops, and the rotation of the trunk ceases. The head and eye movements are thus direct effects of the extirpation of the labyrinth, while the neck and trunk reflexes are secondary and determined by the abnormal position of the head.

The rolling movements are only seen for a short time after the operation, and have been analysed by means of kinematograph records. Each complete turn of the body is due to two attempted movements of progression, accompanied by righting reactions of the trunk which produce the twisting. Since the muscular tonus is diminished on the side of the lesion, the effect of the attempted progression movement is to cause the animal to turn over towards the injured side. The detailed description of the effects of unilateral labyrinthectomy cannot be given here.

(2) Effects of Bilateral Labyrinth Extirpation.—The higher an animal is in the scale, the less important and the more readily dispensed with are its labyrinthine postural reflexes

Experiments on apes and cats show that the effects of double labyrinthectomy are soon recovered from, so that, provided use of the eyes is retained, the animal behaves much as a normal one, except under two conditions.

The first of these is in falling free through the air. It is well known that a cat always falls on its feet, and examination of kinematograph films of falling cats has shown that this is accomplished by the employment of ordinary postural reflexes, the most important of which are labyrinthine ones. During the fall, first the head is righted, then the fore limbs and thorax, finally the hinder part of the body. After

FIG. 107.—Rabbit's skull with oriented magnified models of the utricle and saccule maculæ. Skull in normal position. Stereoscopic. (Magnus, "Körperstellung.")

bilateral labyrinthectomy this power of righting the body during a fall is lost.

The other case in which a response is lost is when the animal is placed in water. Under these circumstances the labyrinthectomised animal fails to orient itself, and would soon drown.

The study of double labyrinthectomy has shown that the animal soon replaces the reflex adjustments which are effected through this channel by others, and it is by the investigation of such conditions that knowledge of the non-labyrinthine responses which have been already discussed has been derived. It is found that the tonus of the muscles is not permanently lowered by removal of both labyrinths,

but is kept up, as Sherrington has shown, by proprioceptive impulses from the muscles themselves, or from the muscles or other structures, such as joints, in other parts of the body, *e.g.*, the neck.

(3) **The Functions of the Otoliths.**—The labyrinth consists of the utricle, into which open the three semicircular canals, and the saccule, which is connected with the utricle by the ductus endolymphaticus. Nerve fibres from the vestibular division of the eighth nerve are supplied to each of the three cristæ acusticæ of the semicircular canals, and to the maculæ of the utricle and saccule. There is one macula in the utricle and one in the saccule. The utricle macula lies in an approximately horizontal position when the head is normally oriented, and the otolith then lies above the hair cells which spring from the macula. The macula of the saccule is situated on the lateral wall of the saccule, and is, therefore, in a vertical position when the head is normally placed, the hairs of its hair cells lying horizontally in the otolith. The exact orientation of these maculæ in the rabbit is illustrated in Fig. 107, which shows a rabbit's skull in the normal position in space, with enlarged models of the maculæ, represented by oval plates, in their proper spatial arrangement, attached to it by wires. The surface of the macula to which the otolith is attached

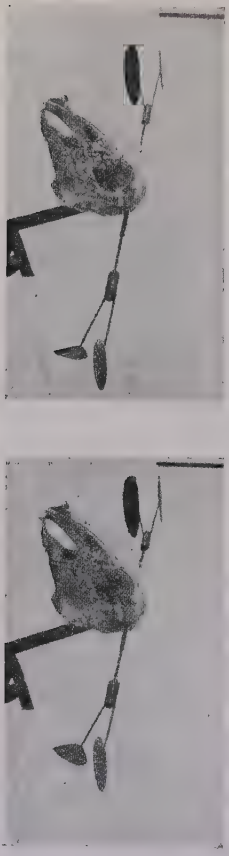

FIG. 108.—Rabbit's skull with maculæ, to show position of head for maximal limb tonus. Stereoscopic. (Magnus, "Körperstellung.")

is indicated in the utricle by white dots, and in the saccule by white stripes on the black plate. Inspection of this figure with a stereoscope shows that the otolith of the utricle lies over its macula, while in the saccule it lies on its outer side.

Now it has been shown by Magnus and de Kleijn that the tonic labyrinth reflex effects on the musculature originate from the utricle macula, while the asymmetric righting reflexes arise from the saccule macula. As regards the symmetrical righting reflexes, it is uncertain whether they arise from one or from both maculæ, though it seems probable that it is from both.

When the animal is placed in the position which gives the maximal labyrinth reflex tonus (p. 348), as shown in Fig. 108, it is found that the utricle otolith is hanging vertically beneath its macula, as in the illustration ; when the head is placed in the position which gives the minimum of limb tonus, then it is found that the otolith is vertically over its macula. Hence it is concluded that the otolith produces the greatest stimulus when it hangs from, and so pulls upon, its hair cells, and the least when it presses them downwards on to the macula. The stimuli are continual so long as the position is kept up, and give rise to steady reflex tonus while they last. Similarly, the saccule macula is least stimulated when the otolith presses upon it, *e.g.*, that on the right side when the left side of the head is below, and most stimulated when the otolith hangs from it, as on the left side when the left side of the head is below. Hence there arise, from the asymmetric nature of the stimuli from the two saccules, asymmetric states of tonus, which constitute the righting reflexes of labyrinthine origin.

The tonic effects of the maculæ in the dogfish have been studied by Maxwell. The results show many points of agreement with those of de Kleijn and Magnus for the rabbit, but there are certain differences, which, however, cannot be discussed in the small space at our disposal.

There is one point which has not so far been touched upon, namely the functions of the semicircular canals in these reflexes. In fishes it appears from the investigations of Maxwell that tonic postural reflexes can also arise from the cristæ of the semicircular canals, but this does not seem to hold good for mammals. Magnus and de Kleijn found that by subjecting guinea-pigs to rapid centrifugalisation it was possible to destroy the otoliths while leaving the semicircular canals intact. When this was done, it was found that all the tonic postural and righting reflexes vanished, while the reflexes to rotation, etc., which are believed to be associated with the semicircular canals, were retained intact. As the functions of the semicircular canals are described in the ordinary text-books of physiology, they need not be further discussed here, though many further details are given in the work by Magnus.

The effects of damage to parts of the labyrinth or of unilateral labyrinth extirpation are all in accordance with the functions which have been previously described above, and also do not need further description in this brief account.

The Nature of Decerebrate Rigidity

The condition of decerebrate rigidity has been described as a caricature of the standing posture, and a study of the causation of the phenomenon is interesting. The researches of Sherrington have made it clear that it is essentially a reflex manifestation; the reflexes responsible for the main phenomenon are proprioceptive, *i.e.*, the afferent stimuli concerned arise in the muscles themselves, though the general postural background thus evoked is capable of definite modifications as a result of afferent stimuli from other parts, such as the neck, labyrinths or skin.

Recent investigations by Liddell and Sherrington have thrown further light on the origin of the proprioceptive

stimuli in the muscles. When any of the anti-gravity muscles are stretched, there results in the muscle a reflex contraction opposing the stretching force. These responses, which are called "*stretch-reflexes*," are strictly localised to the muscle, or the part of it, subjected to stretching, and persist as long as the stretching is continued. There seem to be at least three types of receptor in muscle, and in the perception of posture it is suggested that the Golgi organs of tendon are recorders of tension, while the muscle spindles are probably "length recorders." Both of these when stimulated probably induce reflex contraction. Pain receptors, on the other hand, give rise to reflex inhibitions which are more widespread. The stretch-reflexes provide an adequate explanation of the origin of decerebrate rigidity, for by their operation the action of gravity is effectively opposed.

The Nerve Centres involved in Postural Reflexes

As mentioned on p. 355, the chief centre for the righting reactions is the red nucleus, with the rubro-spinal tract as its efferent path.

(1) **The Cerebellum is not concerned.**—It was shown by Sherrington in 1898 that the removal of the cerebellum does not abolish decerebrate rigidity, and de Kleijn and Magnus have not only confirmed this, but have also shown that the postural reflexes are not in any way altered by removal of the cerebellum and cerebellar nuclei from a thalamus animal, or even from one in which the cerebral hemispheres have been retained. It seems certain that in the simple reflex adjustment of posture the cerebellum is not involved. Though muscular inco-ordination undoubtedly occurs after removal of the cerebellum, it would seem that this only concerns voluntary movements, or else is to be accounted for as due to accidental injury to the labyrinths in the course of the operation.

Recent work suggests that the function of the cerebellum is that of properly co-ordinating voluntary movements by the incorporation of relevant proprioceptor afferent impressions.

(2) **The Position of the Essential Centres.**—By the method of section at different levels of the central nervous system the situations of the chief centres involved in these postural reflexes have been approximately located. They fall into three great groups.

Situated between the level of entry of the vestibular nerves above and the upper cervical cord below are centres which control labyrinth and neck reflexes, excepting the righting reflexes.

Above this level, lying between the vestibular nerve entry and the nuclei for the eye muscles, lie the centres for all labyrinth reflexes on the eyes.

Still higher, in the mid-brain, are the centres for the righting reflexes, that for the neck reflexes also extending down into the pons and upper part of the medulla.

The centres concerned with decerebrate rigidity are situated in the medulla, above the calamus scriptorius. Decerebrate rigidity does not appear if the section of the brain stem is made too far forward, for then it is found that the tonus distribution is normal, and the animal shows righting reflexes. These properties are retained so long as a part of the mid-brain at the level of the large-celled red nucleus is left. Damage of this part of the stem, ventral to the oculomotor nucleus, or of Forel's decussation (of the rubrospinal tract), at once leads to decerebrate rigidity if the pyramidal tracts have also been severed. It is inferred that the red nucleus is the centre concerned in normal tonus distribution and in the righting reflexes. It has been suggested that the spinal centres are under the influence of higher centres of two types: medullary centres, which tend to cause over-action of the extensor muscles, and the red nucleus, which, acting *viâ* the rubrospinal

tract, tends to counteract this by throwing the flexors into action. By the combined action of the two we get in the thalamus animal a normal distribution of tonus; the pyramidal tracts in the intact animal also exert a flexor effect on the spinal centres.

Bazett and Penfield conclude that decerebrate rigidity depends on the integrity of a reflex arc at the level of Deiter's nucleus, and they further show that it persists for two weeks or more, *i.e.*, after the divided long tracts have partly degenerated.

Hemi-decerebration (*i.e.*, on one side only) shows that the tract the section of which gives rise to decerebrate rigidity crosses in the mid-brain (*i.e.*, the rubrospinal).

Muskens has recently thrown doubt on this interpretation of decerebrate rigidity, and considers that it depends upon the severance of secondary and tertiary vestibular connections or nuclei connected with the posterior longitudinal bundle and the posterior commissure.

Postural Reflexes in Man

Although there can be no doubt that when allowance is made for the erect posture the general principles of the stance and righting reactions in man are the same as in the lower animals, and that human posture is an active process of great complexity, yet this is by no means either self-evident or easy of demonstration.

In infants it is possible to show the effect of rotation of the head upon the fore limb stance, and this is also clearly seen in several pathological states in adults. Close examination of stances in athletes or in good statues also reveals many features of interest. Walshe has shown that the tonus of a paralysed limb in hemiplegia is greatly modified by reflexes associated with voluntary movements of the opposite limb, and with the position of the head. The significance of these and other postural reflexes in hemiplegias has been studied by Russell Brain.

Righting reflexes have also been investigated to a small extent. Labyrinthine righting reflexes can be seen in blindfolded children held up by the pelvis, and neck righting reflexes are also shown in many familiar attitudes both of children and adults.

The importance of visual righting reflexes is well known ; the aviator who in a mist may unwittingly be flying upside down, or the Romberg's sign of the tabetic patient are evidence of this.

Deaf mutes with no labyrinths are readily placed in a " zero-condition " should they fall into water ; when they are submerged, visual orientation is lost, tactile orientation is absent and hence no righting reflexes are present, so that they drown if not assisted.

The Peripheral Mechanism of Tonus

The maintenance of a static pose can be continued for many hours or days and yet give rise to no evidences of fatigue, whereas prolonged voluntary contraction of the same muscles is very fatiguing. Many investigations have been carried out with a view to determine the nature of the tonus of voluntary muscles, and to explain the striking difference between phasic and tonic contractions. Of the possible avenues along which an explanation has been sought three might be mentioned. Tonus might be due (*a*) to a different type of metabolism of the muscles, (*b*) to the employment of different muscular structures, or (*c*) to a different type of nerve supply from that employed in the execution of voluntary contractions. It is quite conceivable that more than one, or even all three, differences might exist.

The investigations have been carried out on the most varied material, but the results so far obtained have been rather conflicting.

That the metabolism associated with tonus in the muscles is different from that of voluntarily contracted muscles is

certain, but the difference is quantitative rather than qualitative. It seems certain that in tonus the metabolism of the muscles is little, if any, greater than when at rest; Fröhlich and Meyer, Roaf, and Dusser de Barenne, using various methods, all agree on this point. These facts explain the absence of fatigue. Whether there is even a detectable electrical variation in the tonic muscle has been doubted by several competent workers, though a majority affirm that there is a definite series of electrical variations, as in voluntary contraction, and at the same intervals.

Suggestions that the muscular structures concerned in tonus are not the same as those used in ordinary contraction fall into two categories. One view is that the sarcoplasm of the muscle fibre is concerned with tonus, and the fibrillæ with rapid contraction, the same fibre being thus capable of both types of action. This view may be at once dismissed as lacking any evidence. The other view, which has been advocated by Hunter, is that there are two kinds of fibre in skeletal muscle, the one a red fibre of small thickness rich in sarcoplasm, and capable of tonic contraction, the other an ordinary muscle fibre which executes the usual contractions. According to this view, the muscle is brought by means of the ordinary muscle fibres to the position which it is to occupy and then fixed in that position by means of the fine fibres, which enter into a state of tonic contraction of sufficient power to fix the muscle. According to this hypothesis, the skeletal muscles of vertebrates resemble the muscles of invertebrates in consisting of two kinds of tissue: contractile muscles and tonus muscles. In an oyster, for instance, the shell is closed by the rapid contraction of the striated part of the adductor muscle, but kept in the closed state by the tonic contraction of another part of the adductor which consists of plain muscle. In the skeletal muscle of vertebrates, according to Hunter, both the contractor and tonus muscles are striated, though exhibiting differences in minute structure.

The view that the innervation concerned with the maintenance of the tonic state is different from that which is concerned with contraction began with the demonstration in 1913 by Boeke that some skeletal muscle fibres have a double nerve supply, viz., an ordinary motor supply from the medullated fibres of the anterior roots and a sympathetic post-ganglionic supply consisting of non-medullated fibres which have entered the anterior nerve root by the grey rami communicantes. Since then a number of observers, commencing with de Boer in 1913, have claimed that postural tonus is dependent upon that sympathetic nerve supply.

The double innervation of a proportion of striated muscle fibres must be accepted as proved. Section of the sympathetic fibres leads to degeneration of the sympathetic non-medullated nerve endings in the muscle, while after division of the motor fibres these non-medullated endings of sympathetic origin remain intact. Hunter and Royle have claimed that the fibres to which sympathetic nerve fibres are distributed are the thin "tonus" fibres, and that these receive no ordinary medullated motor fibres; according to them, the tonus muscles are not only different in structure, but have a quite separate innervation. Boeke, however, believes that both kinds of nerve fibres may be distributed to the same muscle fibre.

The experimental evidence advanced in support of the sympathetic origin of tonus is equivocal. Stimulation of the sympathetic supply to a muscle does not induce tonic contraction, neither does the administration of adrenaline (Kuno). The supporters of the sympathetic innervation theory of tonus contend that it would not be expected to do so, since the function of the sympathetic which they claim is that it fixes the muscle at a given length, imparted to the muscle by an ordinary contraction. Their evidence is mainly derived from experiments in which removal of the sympathetic supply to a limb is followed by a reduction of the

tonus of the limb in posture maintenance or in decerebrate rigidity.

Hunter and Royle have made an extensive series of experiments on goats and on birds in which the sympathetic supply of one limb had been removed. In goats, provided the sympathetic supply had been removed several days beforehand, it was claimed that the limb so deprived did not exhibit the firm extensor tonus which the intact limbs do when the animal is decerebrated. Similarly, in birds, removal of the sympathetic supply to one wing resulted in the wing adopting a drooping posture and being more susceptible to fatigue than the control limb.

Dusser de Barenne, van Rijnberk, and Cobb, on the other hand, working on cats, could find no constant effect on decerebrate rigidity by removing the sympathetic chain from one side. Van Rijnberk has also divided the ventral nerve roots to the hind limb, leaving the sympathetic supply intact, and has found this procedure to prevent decerebrate rigidity. Experiments by Forbes and collaborators also failed to show any difference in the decerebrate rigidity of the limbs of cats after sympathectomy. Forbes' able summary of the position of this subject should be consulted for details.

Until better evidence can be presented, therefore, the majority of physiologists seem inclined to reject the idea that tonus is regulated by the sympathetic supply, and to continue to believe that the peripheral structures concerned in tonus are the same as those employed in the execution of ordinary contractions.

The weight of evidence at the present time is unquestionably in favour of Forbes' view that in tonus the fibres are contracting, a few at a time, in relays, each group being relieved by a fresh one before fatigue has set in. The slight degree of fatigue, the small increase of metabolism, and the inconspicuous character of the electrical variations which are observed in tonic states of contraction, are all

compatible with this hypothesis. Moreover, Adrian and Bronk, by methods which are dealt with in Chapter IX., have found that in decerebrate rigidity there is a constant slow stream of nervous impulses (potential waves) passing along the motor nerves supplying the extensor muscles; increase of tonus, or contraction speeds up the rate at which these impulses follow one another.

A suggestion which might possibly explain the function of the sympathetic supply of muscle has come from Orbeli. He maintains that stimulation of the sympathetic, while producing no contraction of the muscle, enables the muscle better to withstand the onset of fatigue; the muscle is thus enabled to sustain its contraction, effected through the usual channels, better than it would do if the sympathetic supply were out of action. Susceptibility to fatigue after sympathectomy is one of the facts which are insisted upon by Hunter and Royle, and it would seem that this aspect of the question is worth following up.

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CHAPTER XII

CONDITIONED REFLEXES

During the nineteenth century the attention of neurological physiologists was mainly directed to the study of the functions of the lower parts of the central nervous system. As a result of the work of Sherrington and others, great advances were made in the study of reflex action, particularly as regards the interaction between different reflexes, as exemplified by synergetic or antagonistic action and reciprocal innervation. The previously fragmentary knowledge of the workings of the spinal cord, medulla, and brain stem was thus welded into a homogeneous and workable chapter of physiology, the practical applications of which to the problems of medicine were not long in following.

The reason for this advance was chiefly that the problem had been studied by properly controlled methods of investigation, such as had already yielded fruitful results in the study of the functions of other organs.

But while our knowledge of the physiology of the lower parts of the central nervous system was, thanks to the application of these methods, growing apace, no comparably rapid advances were made in the study of the higher functions of the cerebral cortex. The reason for this stagnation is not far to seek, for the methods of investigation in current use were anything but physiological, and were capable, therefore, of throwing light on anything except the functions of the cortex. Anatomical and histological researches gave us, it is true, exact knowledge of the structure of the cerebral cortex, and the methods of myelination and degeneration informed us as to the possible paths of connection between different

portions of the brain and the lower parts of the central nervous system. Also, electrical excitation of the cortex had led to further anatomical localisations of particular functions. But all these methods told us nothing of the manner of working of the brain. Psychological methods based on introspection, together with a statistical study of animal behaviour, represented almost the only lines of attack on the question of function, and these methods, owing partly to their lack of connection with the already established facts regarding the physiology of the lower parts, and partly to the difficulty of proper control, led rather to the construction of elaborate technical vocabularies than to physiological explanations of function. In consequence, we find in physiological text-books a remarkable paucity of information concerning the functions, as distinct from the mere structure, of the cerebrum.

The difficulties of investigation have their root in the complexity of the reactions of an animal endowed with cerebral hemispheres when compared with those seen in the spinal animal. In the latter the responses to particular kinds and strengths of stimuli can be predicted, and when no stimuli are applied, the animal remains completely inert. In the normal animal, on the contrary, the reflex activities seem never to repeat themselves in the same way either as regards their strength or their character. It is impossible to say which stimuli will be followed by a response or what the nature or intensity of the response will be. Very often, indeed, the animal exhibits activities evoked by no apparent stimulus, these being to all appearance what the psychologist would call "voluntary actions." Evidently, then, it is impossible by mere observation under ordinary conditions of life to formulate any rules for the appearance of such reflexes.

As a result of many years of persistent work, Professor Pavlov and his numerous pupils have evolved a trustworthy method for the study of the higher reflexes in animals and in

man. The method is entirely physiological and quite objective, depending neither on the subjective methods of the psychologist nor on the findings of purely anatomical methods, the results of both of these means of approach being accessory to the study, and not ends in themselves. These studies have shown that the complexity of the actions of the cerebral hemispheres is to a great extent more apparent than real. As a matter of fact, its functions are of truly reflex type, but are subjected to inhibition as a result of many extraneous circumstances, and it is the intrusion of a great variety of such inhibitory influences which makes the workings of the cerebral hemispheres at first sight appear to be so arbitrary.

The Inborn and Acquired Reflexes

All the reflex activities of animals can be divided into two large groups. The first group consists of reflexes which are transmitted by heredity, and which are the characteristics rather of the species of animal than of any individual animal. They appear in every member of each species of animal provided its central nervous system is normal. They may disappear with age, or develop only in maturity, but always to reappear and follow a similar course in the next generation. All the purely spinal, medullary, and cerebellar reflexes are included in this group. Simple reflex activities of the type of the knee-jerk, the corneal reflex, and complex reflexes, like those concerned in the maintenance of equilibrium, the stepping reflex, vaso-motor reflexes, or still more complex reflexes, or rather combinations of successive and simultaneous reflexes, like the lower sexual reflexes, alimentary reflexes, and so forth, reflexes which are often called by biologists by the indefinite name of "instincts," all belong to the first group of inborn or, as Pavlov preferred to call them, *unconditioned* reflexes. The latter name is used simply to denote the extreme regularity of these reflexes and

their independence of, or slight dependence upon, the surrounding conditions.

Besides this type of inborn or unconditioned reflexes, each particular animal presents a large number of reflex activities which are the individual properties of its own central nervous system, and are not necessarily found in any other representatives of the same species. Every dog secretes saliva when food is placed in its mouth, and performs definite and co-ordinated muscular contractions when it is injured by a stimulus. But an individual animal may also secrete saliva when it sees the particular man who feeds it, or the plate from which it is usually fed. It will often run away long before the stimulus causing injury has been applied. The mere sight of the instrument by means of which injury has once been inflicted will be sufficient to produce in it the same reflex activity as the injury itself would cause in the spinal animal.

The individual reflexes may be restricted to one single animal, to a small group of animals, or to the entire species. In the latter case special methods must be applied in order to determine whether that particular reflex is of the inborn type or whether it has been acquired after birth. All reflexes of the latter type are acquired individually; they are the achievement of each and every central nervous system during its own existence, and are an expression of the experiences of each individual animal. The acquired reflexes Pavlov calls *conditioned* reflexes, in order to denote their very large dependence on the surroundings and upon the conditions of their establishment. Conditioned reflexes may be of a very simple nature, or they may form groups and thus present compound reflexes which may be even more complex than the most complex unconditioned reflexes.

The method of investigation of conditioned reflexes consists in the experimental development and study of acquired reflexes. Normal animals respond only to some of the stimuli to which they are subjected, and it is impossible to

predict which of the stimuli will produce a reflex and which will not, whereas the spinal animal is an automat. The new method of investigation aims at the building up, in the normal animal, of reflexes which can be evoked with the same certainty as the spinal reflexes in a decapitated animal.

Conditions which are necessary for the Formation of Acquired Reflexes

The first and the most important principle involved in the development of conditioned reflexes is that they develop only on the basis of another already existent reflex, which may be an unconditioned, or may be another conditioned, reflex. There is never spontaneous development of conditioned reflexes. The law of formation of conditioned reflexes is that *stimulation of any receptor organ occurring simultaneously with a reflex excitation of an effector organ leads to formation of a new reflex.*

It is necessary that a stimulus which has been chosen for the purpose of developing a conditioned reflex be administered simultaneously with another stimulus which already produces a reflex activity in one or another effector organ. An example will illustrate this statement. A knock on the knee results in the knee-jerk. If another stimulus, *e.g.*, a visual stimulus, is administered during the time of the occurrence of the knee-jerk, and the simultaneous action of the two stimuli is repeated for a sufficient number of times, it will be found that the formerly neutral stimulus will ultimately elicit the knee-jerk when applied by itself; it has become a *conditioned stimulus* (Fig. 109). Different receptor organs can be used for the establishment of conditioned reflexes, and different effectors can be used for the same purpose. Conditioned reflexes have been established with respect to the vaso-motor system, the heart, the pupils, muscular movements, the secretion of different glands of the alimentary tract, expulsion of milk, and micturition. In

all cases the underlying principle of the formation of the new reflexes is the same. If a given receptor organ is stimulated

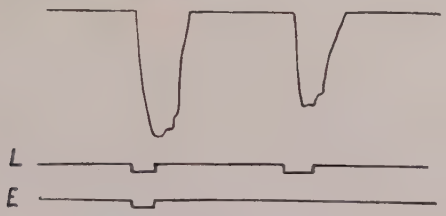

FIG. 109.—The knee-jerk as a conditioned reflex. L = application of the conditioned stimulus, which was a flash of light; E = the unconditioned stimulus, applied to the patellar tendon. In preparing the reflex the two are simultaneous, as at the first signals. Ultimately the light stimulus will act alone, as at the second signal. (Protopopov. Dissertation, 1911.)

during an occurrence of any of these originally unconditioned reflexes, a new link, a new nervous path, is formed between

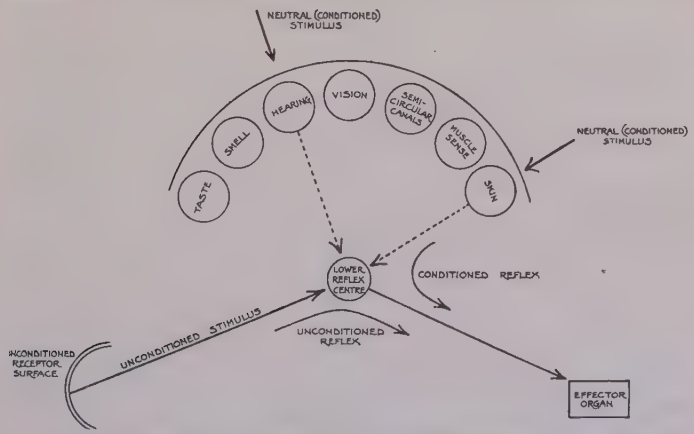

FIG. 110.—Diagram to illustrate the establishment of conditioned reflexes. Two conditioned reflexes are shown, one for hearing and one for skin stimuli.

the new receptors and the effector organ (Fig. 110). A stimulus which previously had never produced any reflex

activities in the animal has now acquired the properties of the unconditioned stimulus upon which it is based.

Most of the investigations upon conditioned reflexes on dogs were performed with the parotid salivary gland as the effector organ, the reason being that this gland is to a large extent independent of the activities of other organs and gives a single type of response (secretion). Further, a salivary fistula can easily be established without subjecting the animal to abnormal conditions, and the responses can be quantitatively measured by recording the number of drops of saliva secreted. Glandular structures have no tone, and the registration and measurement of the reflex is simpler than would be the case in muscular reflexes. The table below illustrates such a formation of a new salivary conditioned reflex. A sound of definite character was used as the conditioned

Development of a Conditioned Reflex to a Sound of 637·5 Vibrations per Second.
(Anrep : *Journ. of Physiol.*, 1920.)

Number of times Combination of Sound with Feeding had been performed.	Extent of Reflex in 30 seconds, number of drops of saliva.	Latent Period of the Reflex, seconds.
1	0	—
9	18	15
15	30	4
31	65	2
41	69	1
51	64	2

stimulus. When applied before the conditioned reflex had been formed, it, of course, did not produce any secretion of saliva. After several applications of this sound, together with feeding of the animal, the sound itself acquired the same properties as food in respect to the salivary glands. The reflex grew in force, the latent period shortened, and finally the response became constant. The fundamental condition for

the formation of conditioned reflexes of this description is the *simultaneous* application of the originally neutral stimulus and of any other stimulus which elicits *ordinary reflex activity*. Hence these are called *simultaneous conditioned reflexes*.

But the two stimuli may also be applied in a different order. Thus the unconditioned stimulus (*e.g.*, food) may precede the neutral stimulus (the sound). In this case conditioned reflexes do not develop at all. The interval between, say, the presentation of food (the unconditioned stimulus) and the application of the neutral stimulus may be only a few seconds, but this is sufficient to make the formation of the conditioned reflex impossible. But alternatively the conditioned stimulus may precede the unconditioned, and now the reflex is firmly established after a few repetitions of the two stimuli in this order of sequence. The time interval between the beginning of the first and that of the second stimulus may be varied to a very great extent—from a few seconds up to many minutes—but in all such instances the conditioned stimulus is continued throughout the interval. When developed under such conditions it is found that the latent period of the conditioned reflexes is equal to the time interval between the two stimuli. The appearance of the reflex may be practically simultaneous with the application of the conditioned stimulus if the time interval used has been very short, but the latent period will be long when the time interval was long. Conditioned reflexes of the latter type are generally known as *delayed conditioned reflexes*.

By another method the conditioned stimulus also precedes the unconditioned, but the latter is not applied until after a pause. In this case the conditioned stimulus is kept on for some time and then stopped, and the unconditioned stimulus is presented only after a certain definite interval of time. In instances of this type it is not the actual conditioned stimulus, but merely its trace left in the central nervous system, that is associated with the unconditioned stimulus.

This type of reflex has also a latent period equal to the interval of time between the beginning of the conditioned and that of the unconditioned stimulus. Reflexes of this kind are known as *trace reflexes.*

The Rate of Formation of Conditioned Reflexes

Simultaneous conditioned reflexes are in every case formed much more quickly than reflexes of the delayed or trace kind. Conditioned reflexes in response to cutaneous, auditory, and olfactory stimuli develop in dogs, as a rule, much sooner than reflexes to thermal stimuli. To a large extent the rate at which conditioned reflexes become established depends upon the strength of the conditioned stimulus which is employed. A very good example is given by the study of conditioned reflexes in which cutaneous tactile or thermal excitations form the conditioned stimuli. A very weak stimulus applied to either of these types of sensory receptor will lead to a formation of the conditioned reflex after, say, fifty or sixty applications. A stronger stimulus will cause a formation of the reflex in a much shorter time. But a very strong stimulus, again, delays the formation of the reflex.[1] It seems that for each variety of receptor organ there is an optimal strength of stimulus at which the reflex most quickly develops.

Provided all external conditions are maintained rigidly constant, the vigour of the conditioned reflex depends upon the strength of the reflex upon which it has been established. If the unconditioned stimulus produce so weak an effect as to give only a feeble reflex response, then a conditioned stimulus can only establish on its basis a reflex which is similarly weak, while, whatever the strength of the conditioned stimulus itself may be, conditioned reflexes which

[1] This is probably because very powerful stimuli, though neutral with regard to the salivary glands, are not neutral with respect to other effectors and evoke unconditioned reflexesof various types ; *e.g.*, strong tactile, visual or thermal stimuli will bring about defensive reflexes characteristic of pain.

are based upon strong unconditioned ones will be powerful and will tend ultimately to attain a strength similar to that of the unconditioned reflex. Discontinuous conditioned stimuli lead to a more rapid formation of conditioned reflexes, which are generally also of a somewhat stronger type than those established by means of continuous stimuli.

Properties of Conditioned Reflexes

(1) **Summation.**—If a conditioned reflex has been established to the application of, say, sound, and another conditioned reflex to, say, a cutaneous stimulus, both reflexes having, as in Fig. 110, the same effector path, the simultaneous application of both conditioned stimuli will result in a more powerful reflex activity. Thus in one series of experiments the smell of camphor had been made a conditioned stimulus for secretion of saliva, and this stimulus gave a secretion of 60 drops. In the same animal, electrical stimulation at a given place on the skin had also been worked up as a conditioned stimulus, giving a secretion of 30 drops. When the two stimuli, smell of camphor and electrical skin stimulation, were applied simultaneously, the animal secreted 90 drops of saliva in the same time.

Sometimes the summation is, as in the instance just given, a quantitative one, though more often the compound stimulus merely gives a somewhat stronger reflex than that given by either of the two stimuli separately. It is found that summation is much more likely to be quantitative if the two reflexes concerned are elicited from different receptors, as in the instance quoted, than if the conditioned stimuli are both applied to the same receptor, *e.g.*, two different sounds or two lights of different intensities.

(2) **Specificity.**—A conditioned reflex established to any given stimulus does not lead to formation of other conditioned reflexes, so that in the case of the experiment given in the table on p. 378, for instance, the casual application of a

visual stimulus, stimulation of the skin or of any other receptor organ, will not lead to secretion of saliva. Within one given receptor organ the specificity of conditioned reflexes to *similar modes* of stimulation is not so well marked. If a reflex is established to a tone of, say, 800 d.v., a sound of 1,000 vibrations will also produce an effect, but only on a much smaller scale, though there would be no response at all to, say, the beat of a metronome or a blast on a whistle. As the conditioned reflex becomes more and more established its specificity increases, so that with further repetitions of the combined stimuli the range of accessory reflexes due to similar, but not identical, stimuli is narrowed.

(3) **Stability.**—In order to maintain the strength of conditioned reflexes constant, they must be regularly and repeatedly "*reinforced*" by the unconditioned stimulus. Isolated application of the conditioned stimulus without the unconditioned should, therefore, either be avoided altogether or should be performed as rarely as possible; otherwise the conditioned reflex weakens. If work with any particular reflex has been interrupted for even a long period of time—several months or more—and is then resumed, it is found that the mere interruption has not destroyed the conditioned reflexes. Though weakened, they will still be present and can be brought to their original strength in a much shorter time than would be required for the development of new reflexes.

(4) **Experimental Extinction.**—If the principal rule for the formation and maintenance of conditioned reflexes is violated, *i.e.*, if a conditioned stimulus is applied for several times in succession without the accompaniment of the unconditioned stimulus, the reflex undergoes rapid diminution in strength, and can in this way quickly be extinguished. The shorter the interval between the isolated applications of the conditioned stimulus, the quicker will be the extinction (Table, p. 383). Delayed and trace reflexes undergo in every case a much more rapid process of extinction than the

(Babkin. Dissertation, 1903.)

Experimental Extinction of Conditioned Reflex.

The conditioned stimulus was given without being followed by the unconditioned.

Intervals between successive applications of conditioned Stimuli.	Time necessary for Extinction of Reflex.
2 minutes . . .	15 minutes.
4 ,, . . .	20 ,,
8 ,, . . .	54 ,,
16 ,, not extinguished in	120 ,.

corresponding simultaneous reflexes. A conditioned reflex which has undergone such extinction, however, regenerates spontaneously after an interval of a few hours, and without any reinforcement by the unconditioned stimulus. Repeated extinction has much more profound effects, and the spontaneous regeneration of such reflexes is progressively slower and less complete, so that after having undergone several extinctions the reflex disappears altogether. When this has happened, it may take a longer time to re-establish the reflex than even for the formation of a new reflex. The study of experimental extinction of conditioned reflexes reveals a fact of primary importance, namely that extinction of a conditioned reflex for a particular stimulus from one receptor has no effect upon the strength of any conditioned reflexes from other receptor organs. Its effect even upon the strength of the conditioned reflexes to other stimuli from the same receptor is either absent or suffers only a temporary reduction, and in the latter case always very small. This shows that each conditioned reflex is an isolated physiological entity, both as regards its inception and its extinction. Unconditioned reflexes on repetition undergo practically no diminution in their strength.

Irradiation of Conditioned Reflexes

Conditioned reflexes, though specific as between different receptor organs, are only to some extent specific within one

single receptor organ, and in this respect reflexes of the simultaneous, delayed, and trace types present several peculiarities. For simplicity we will refer to the following example. A tactile conditioned reflex is formed from any given place on an animal's skin, the application of the cutaneous stimulus preceding the feeding by only a few seconds. After this (practically simultaneous) reflex has been

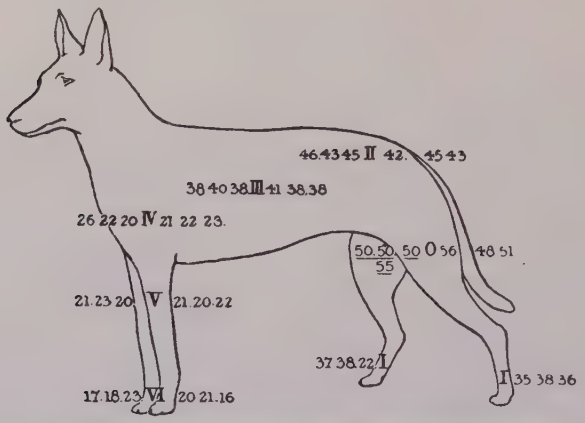

FIG. 111.—To illustrate the irradiation of conditioned reflexes. O and Roman figures I., II., etc., indicate the places stimulated, O being the original site of stimulus; Arabic figures to left of these = drops of saliva by stimulating that spot on animal's left-hand side; Arabic figures to right = result of similar stimuli applied to right side. (Anrep, *Proceedings of the Royal Society.*)

established it will be found that similar stimulation applied to other parts of the skin will have a much smaller effect than that obtained from the original area. As the work proceeds, the area of irradiation of the reflex is narrowed to a small patch around the original position. In this case the reflex is nearly specific within the same receptor organ, and finally shows hardly any sign of irradiation. Reflexes of the delayed type, however long the latent period may be, always culminate in such strictly localised reflexes. In the case of trace reflexes, the irradiation of the reflex is much wider,

so that if a cutaneous conditioned reflex of this type is formed for any particular region of the skin it may be found that stimulation of any other part of the skin will give a reflex of a strength identical with the original. The longer the latent period of a trace reflex, the wider its irradiation. With trace reflexes of very short latent period (three to five seconds) the irradiation is only partial, the places nearer to the place of original formation of the reflex producing a stronger response than the places further away. An interesting fact has been observed by Anrep in the case of cutaneous conditioned reflexes, viz., that the skin of the contra-lateral side behaves in a manner identical with that of the side on which the reflex was originally formed, symmetrical places giving the same strength of response (Fig. 111). In the case of trace reflexes with a long latent period, the irradiation may affect not only receptor organs of the same type, but even other receptor organs, so that in this case the conditioned reflex entirely loses its specificity and becomes generalised in respect to all receptors. The latent periods of all these accessory irradiated reflexes will be the same as that of the original conditioned reflex. Conditioned reflexes of this type, but formed in other receptor organs, behave according to exactly the same rule.

Discrimination

It has been stated that a single conditioned reflex can be extinguished without producing the slightest effect on any other reflex. It has also been shown that the specificity of conditioned reflexes even within one single receptor is not perfect. A reflex to a sound of 800 vibrations per second will lead to development of accessory reflexes to about 200 or 300 vibrations per second above and below the original stimulus. If, however, any such accessory stimuli are applied to the dog without being accompanied by the unconditioned stimulus, they undergo quick experimental

extinction, the original reflex not being affected, and therefore becoming more specific. By this method of contrasting the two stimuli it was possible to study in an objective manner the power of animals to discriminate between different stimuli (Fig. 112). It was possible to show that animals have powers of discrimination different from those in man, and also to study the limits of this discrimination. By the use of the method of experimental extinction the animal develops, through its experience,

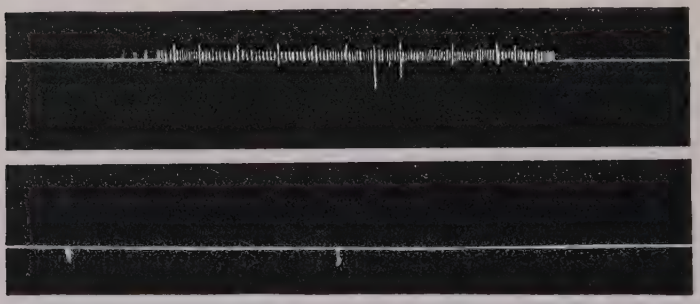

FIG. 112.—Discrimination of stimuli in conditioned salivary reflex. Upper tracing = reflex secretion of saliva in response to note of 637·5 vibrations, lasting thirty seconds; each mark = 0·01 c.c. saliva; lower line = no response to note of 680 vibrations for thirty seconds. (Anrep, *Journal of Physiology*, 1920, **53**.)

not only new conditioned reflexes, but also new states of inactivity of the central nervous system. The strength of the stimulus in this respect plays only a minor part, and it is possible to use a very weak stimulus to produce the conditioned reflex, while a similar strong stimulus may be extinguished and will not result in a reflex activity. In this respect the conditioned reflex differs in a striking manner from the *unconditioned*, in which the strength of the response is always proportional to the strength of the stimulus.

The central nervous system, therefore, possesses not only the highly important function of the development of new sets of reflexes, which may be called its synthetic activity, but

also of the discrimination between very closely related stimuli, differentiating these into effective and ineffective. The latter side may be called its analytical activity. Pavlov for this reason introduced the name of *analyser* for the entire mechanism comprising those parts of the central and peripheral nervous system which are related to each particular receptor organ. Though no doubt the main analysis occurs in the cortical parts of the analyser, the determination of the exact location of the analysis must be left to further researches.

The Analysers

(1) **The Auditory Analyser.**—By the method of conditioned reflexes it was possible to show that a dog hears sounds up to 120,000 vibrations per second, which is much higher than the limit of audibility for any human ear. Discrimination of pitch is also very much more developed in the dog than in man, differences in pitch of one-eighth of a tone being perceived by this animal. The direction of sounds, as well as their strength and timbre, are perceived by the dog with much greater precision than by man.

Recent work on specially operated animals has thrown light upon the long-debated theory of hearing of Helmholtz. Partial destruction of the organ of Corti on one side, with complete destruction of that on the other side, led only to the disappearance of conditioned reflexes to a certain range of vibration frequencies. In some dogs the reactions to high-pitched notes were absent, while the reactions to others were normal. In others the middle scale entirely disappeared. In both cases the result depended on the site of the lesion in the partly damaged cochlea.

(2) **The Visual Analyser.**—Light and darkness, different shapes of objects, *e.g.*, letters, the positions of the objects, particular movements, rotations of objects in different directions, perpendicular and horizontal movements, special rates of movement, different degrees of luminosity, could all

be used as conditioned stimuli for the formation of reflexes, and quite slight modifications in the stimuli could be discriminated by the animals. Dogs and cats could not, however, be made to discriminate colours if these were of the same luminosity. The discrimination of visual stimuli is in every respect much less perfect in the dog than in man.

(3) **The Cutaneous Analysers.**—Conditioned reflexes established to tactile stimuli are well differentiated by dogs from other kinds of stimuli, and also from cutaneous thermal stimuli. There is also differentiation between different tactile stimuli. For instance, the application of a rough surface can easily be discriminated from that of a smooth surface, and the strength of a pressure and the duration of a stimulus are also easily discriminated. In the case of the thermal receptors the limit of discrimination is reached at about $\frac{1}{2}^\circ$ to 1° C. difference of temperature, and varies according to the difference between the stimulating temperature and the animal's own skin temperature.

(4) **Other Analysers.**—Conditioned reflexes have also been established to smell, to different stimuli applied to the taste buds, to the semi-circular canals, or to other proprioceptors, as for instance those of muscle sense. Pain could be used for the conditioned stimulus, and it was possible to establish the food reaction to such stimuli as ordinarily produced only muscular movements of defence. The pain stimuli could even be made discriminative, so that stronger stimuli would give an alimentary action because they had become associated with feeding, while weaker and less painful stimuli would give the normal muscular reflex response.

The Inhibition of Conditioned Reflexes

External Inhibition.—If during or shortly before the occurrence of a conditioned reflex the central nervous system has been affected by any kind of stimulus, which need not

necessarily have given rise to reflex activity of any kind, it will be found that the conditioned reflex has become much weaker. The stronger this additional (or " extra ") stimulus, the greater is its inhibitory effect. However, this inhibition is only temporary, and if the extraneous stimulus is repeated, its inhibitory effect will become progressively smaller, and finally will disappear altogether. This type of inhibition is not an acquired property of the central nervous system, and does not involve any development of an adaptation on the part of the animal. The phenomenon is known as *external inhibition* (Table below). Very brief extraneous stimuli, sometimes even those

External Inhibition by Extra Stimulus.

The conditioned stimulus was a visual one. The extra stimulus was provided by playing a gramophone record for a few seconds. At first there is external inhibition, but it disappeared rapidly on repetition of the extra stimulus.

Strength of visual conditioned reflex			=	100	per cent.
Effect of gramophone,	1st	application	=	10	,,
,, ,,	2nd	,,	=	50	,,
,, ,,	3rd	,,	=	65	,,
,, ,,	4th	,,	=	85	,,
,, ,,	5th	,,	=	90	,,
,, ,,	6th	,,	=	94	,,
,, ,,	7th	,,	=	100	,,

imperceptible to man, can thus affect the strength of conditioned reflexes and introduce a serious disturbing element into the experiments. It is, therefore, imperative to use special precautions to maintain the condition of the animals under experiment perfectly constant and controlled. For this purpose special laboratories have been built. The experimenter is always outside the room in which the animal is placed, and all connections between the worker and the animal—for instance, the administration of food (the unconditioned stimulus), registration of the salivary

secretion, and administration of the conditioned stimuli—are performed from the outside of the animal's cell (Fig. 113). Only by such precautions is it possible to achieve perfectly stable conditioned reflexes which will not exhibit arbitrary fluctuations of intensity.

Internal Inhibition.—Besides the external inhibition, there are other types of inhibitory influences which differ from it in many respects. We have already met with several conditions of the animal under which a stimulus which was

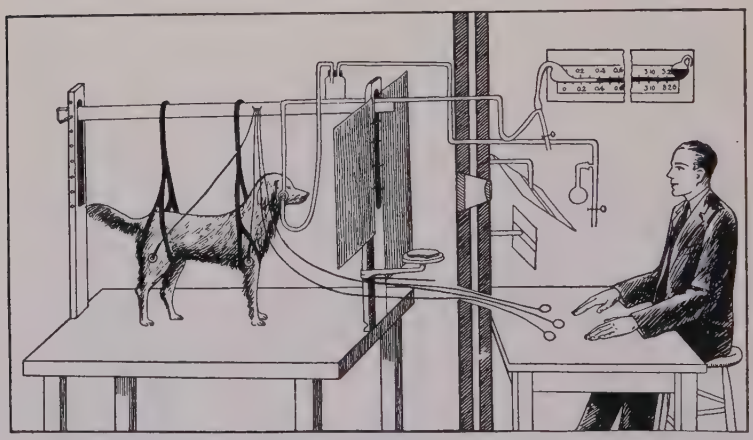

FIG. 113.—Operator outside the cell observing animal by periscope; also shows the various controls. (*Pavlov.*)

previously effective becomes ineffective as a result of certain adaptations on the part of the animal. For instance, in the experimental extinction of conditioned reflexes a reflex of 100 per cent. strength can be reduced to zero in a few minutes. In the discrimination of stimuli also, reflexes which were not at first discriminated can be differentiated after the application of the method of contrasting the two stimuli, one of which now becomes ineffective. Again, with trace and delayed reflexes the effect of the stimulus remains in abeyance for a certain time, *i.e.*, during the latent

period. All these states of temporary inactivity are the result of adaptation of the animal to the changes in its surroundings. It may be presumed that these states of inactivity are the result either of the development of an inert condition of the animal to any of these ineffective stimuli, or of fatigue, as, *e.g.*, in extinction, or of the development of an active inhibitory process. All the experimental evidence demonstrates that the latter presumption is the correct one. They have, therefore, been called *internal inhibitions*. The evidence given here that the process is inhibitory will be, first, the study of the effects of extra stimuli upon these states of inhibition, and, second, the after-effect of conditioned reflexes.

The Effect of External Inhibition upon Internal Inhibition.— We have seen that an extra stimulus applied during or before a conditioned stimulus results in a diminution of the strength, or in abolition, of the conditioned response. But the same extra stimulus, when applied together with, say, a sound stimulus of a pitch which has been rendered ineffective by discrimination from an effective sound stimulus, results in secretion of saliva. This is because the extra stimulus has inhibited an already present state of inhibition and thus revealed the primary conditioned reflex. There are many other well-known examples in reflex phenomena of the emergence, as a kind of release, of a reflex by the suppression of an inhibition which held it in check. Extra stimuli applied even after total extinction of the reflex also result in the reappearance of the reflex. When applied during the latent period of a prolonged trace or delayed reflex, the inhibition is again removed, and the primary inhibited reflex emerges prematurely. The effect of external inhibition is thus antagonistic to any of the mentioned cases of developed inactivity of the central nervous system. To all these cases of developed inactivity Pavlov gives the name of "*internal*" *inhibition*. Internal inhibition does not differ in any respect from any ordinary conditioned reflexes except

in being a state of acquired inactivity instead of activity. Different internal inhibitions may undergo summation ; they can be experimentally extinguished in the same manner as conditioned reflexes, the stimuli causing inhibition can be discriminated from each other, and they can in every respect be called *negative conditioned reflexes.*

The After-effect of Conditioned Reflexes.—Both the inhibitory and the excitatory conditioned reflexes have certain after-effects. These after-effects can be studied in any analyser, but it has been found much easier to perform the experiments on the cutaneous analysers. One conditioned reflex is established at a certain place on the skin. Another place on the skin is also frequently stimulated with the same stimulus, but never combined with food. The result is that the first leads to development of the positive conditioned reflex, the other to a negative conditioned reflex. The one stimulus signifies "food," the other one "no food." The positive conditioned reflex is almost always of the same strength, but if tried a very short time after the application of the negative conditioned stimulus, it will be found that the strength of the positive reaction has been reduced. The shorter the interval of time that has elapsed since the administration of the " negative " stimulus, the greater will be the reduction of the positive reflex. Also, the inhibitory after-effect of the " negative " stimulus will disappear with a freshly established reflex much more slowly than with a firmly established one. The inhibitory after-effect of stimuli at other places is much more pronounced when these are near to the seat of induced inhibition, and they may have hardly any effect at situations far away. In freshly established reflexes the inhibitory after-effects may spread from one analyser to another, so that a conditioned reflex of the auditory or visual type will often thus get partially inhibited by a negative conditioned reflex from, say, the cutaneous or other areas.

Inhibitory after-effects readily summate, so that the

after-effect which lingers after several applications of the inhibitory stimulus will be much more profound and will persist for a much longer time than that resulting from a single application. The area of effectiveness of the inhibitory after-effects will then also be greatly extended, so that it will probably include most of the analysers of the animal.

The strength of the inhibitory after-effect also depends on the intensity of the inhibition primarily set up in the brain by the inhibitory stimulus. If a certain musical note is discriminated by the animal from notes one-eighth, one-half, and one full-tone higher or lower in pitch, the application of the note which is one-eighth higher in pitch will always have a much more pronounced inhibitory after-effect than that left by the application of a full-tone difference.

On careful examination of the rate at which the inhibitory or excitatory processes spread in the brain it was found that a certain time is needed for the full development of a particular effect arising from a given place. With the cutaneous analyser, the application of an inhibitory stimulus at one place will lead to the after-effect being developed to a maximum at another place within thirty to sixty seconds. After that the inhibitory after-effects will become smaller and smaller. Several explanations have been advanced for this phenomenon of the gradual development of the inhibitory after-effect. Recent experiments tend to show that the most probable explanation is the following. During the process of transmission of an inhibitory stimulus to one part of the brain, the rest of the organ undergoes a state of induced hyperexcitability. Conversely, with stimulation the rest of the cortex falls into a state of induced inhibition. The spread of the inhibitory process, therefore, meets a state of hyperexcitation which it has to overcome. The induced excitability diminishes rapidly after the termination of the inhibitory stimulus, and what at first seems like a gradual increment in the inhibitory after-effect is in reality the result

of the two antagonistic processes proceeding at the same time.

The Seat of Inhibition.—Different analysers can be used, one for the excitatory, another for the inhibitory, stimulus, so that the application of the excitatory stimulus alone gives a 100 per cent. reflex, while when applied together with the other stimulus it gives no reflex activity at all, being inhibited by the second stimulus. The question is whether the inhibition primarily develops in the excitatory analyser or in the analyser which is used to inhibit the former. Several observations point to the fact that the inhibition originates within the excitatory analyser. If a cutaneous reflex is capable of inhibition by an auditory stimulus, the inhibitory after-effect will be much more marked in the cutaneous analyser than in the auditory or any other one, which shows that the cutaneous analyser was the point of origin from which the inhibition has spread to the rest of the cortex. It might be conceived that the inhibition affects not the cortical areas, but the subcortical centres of the effector organ under investigation. However, the following experiments show that this is incorrect. The salivary conditioned reflex is established to a cutaneous stimulus, and an auditory stimulus is used to inhibit it. The inhibition and the reflex itself are well developed, and, as shown before, if work is interrupted for a couple of weeks, neither the reflex nor the inhibition is much reduced in precision. During this interval of time the same cutaneous stimulus is used for establishment of a new reflex, the effector organ of which is another than in the case of the first reflex, *e.g.*, contraction of a muscle instead of secretion of saliva. When this reflex has been thoroughly established, the excitatory (cutaneous) stimulus is now again applied in combination with the sound-stimulus which inhibited the original reflex, and in every case it also produces the inhibition of the new reflex. This shows that the seat of inhibition cannot be within the lower central nervous system, because both effectors, and therefore

their central connections, had only one common path, namely in the cortex.

Conditioned Reflexes of Higher Orders

So far we have only described those conditional reflexes which are established on the basis of an inborn or unconditioned reflex. Conditioned reflexes can, however, be established also on the basis of other conditioned reflexes, and this refers to both the excitatory and inhibitory types of response. The following examples illustrate this. A stimulus, A—say, a sound—is made to be a conditioned stimulus, so that whenever A is applied to the animal a 100 per cent. reflex occurs. Another stimulus, B—say, a tactile stimulus—is used to inhibit the excitatory stimulus, so that when A is used together with B no reflex occurs. Now for several times in succession the inhibitory stimulus B, which by itself does not cause any secretion, is used with a third stimulus, C—say, a flash of light—which also, when tested before, had neither inhibitory nor excitatory properties. After several applications of C and B together, C is used in combination with A, and it is found that A is now inhibited by the stimulus C. This means that a stimulus to which the animal was previously quite indifferent has acquired the properties of the inhibitory stimulus when used in association with the latter. In this manner conditioned reflexes, negative and positive, can be established, but it has been found that animals as low as dogs could not develop conditioned reflexes beyond the second, or sometimes third, order, though in human beings it seemed that this type of conditioned reflex can develop without discoverable limit.

A neutral stimulus therefore acquires the characters of any stimulus the effects of which frequently predominate in the central nervous system at the time of its application. If the neutral stimulus habitually falls within a period when inhibitory processes predominate, it acquires inhibitory properties, and the same is true with excitation.

Localisation of Conditioned Reflexes

Effect of Total Extirpation of the Cortex.—Nearly complete extirpation of the cortex was first performed by Goltz. The animal survived for several months, but comparatively little knowledge was gained by mere observation of such an animal, whose behaviour was generally described as that of an automaton. The main conclusion was that animals thus operated upon do not form any new associations, and lose those which they had previously formed. Whether or not they lost audition, vision, taste, and other senses remained matters for dispute. Munk ascertained that after extirpation of cortical areas, although the animals lost associative memory, they were capable of hearing and seeing. Other experimenters found in the brain associative areas and higher associative centres, which they located generally in the frontal and occipital areas. The extirpation of those areas rendered the animals powerless to form associations. The method of conditioned reflexes has given a reliable way of determining the changes occurring after extirpation of different areas.

After total extirpation of the cortex in dogs, which survived up to four years and eleven months, it was found that no conditioned reflex potentialities remained, though all the lower reflexes were normal. Administration of food still caused salivary and gastric secretion. The lower sexual reflexes and self-preserving reflexes were retained, but none of these could be used for the establishment of conditioned reflexes ; the animals reacted to sounds and to different tastes. Localised damage to cortical areas has apparently only a temporary effect upon conditioned reflexes, the inhibitory processes being affected to a much larger extent than the excitatory, so that experimental extinction, differentiation of stimuli, and delayed reflexes are re-established with greater difficulty. After a few months these differences mostly disappear, and the animal is normal, all conditioned

reflexes being again present. More extensive damage to different areas of the brain causes a permanent weakening of the inhibitory processes, though the conditioned reflexes themselves are still present. All these experiments demonstrate the fact that vicariation of functions is developed to a large extent within one single analyser. After extirpation of the whole anatomical cortical area corresponding to an analyser all conditioned reflexes from that particular receptor are permanently lost. This has been studied for the auditory, visual, tactile, and other analysers. In most cases the area found by means of conditioned reflexes corresponds to the anatomical area, though generally the former is somewhat more extensive. One exception is the pyriform lobe (of which the uncus in man is the small vestige); after extirpation of this convolution all the animals still retained power to react to, and to discriminate between, smells. Extirpation of the motor area on both sides resulted in a comparatively rapid recovery of most of the lower reflexes, though conditioned reflexes from muscle sense could not then be re-established. On the basis of these experiments, performed on many animals, Pavlov believes that the motor area has exactly the same function in respect to muscle sense as other areas in respect to their corresponding sense organs. A striking fact has been found with inhibition. After bilateral extirpation of the cortical area corresponding to one whole analyser this analyser can never be used again for the establishment of the conditioned reflex, but can be successfully used for *inhibition* of other conditioned reflexes, which once more shows that the seat of inhibition is within the inhibited analyser, and not within the inhibiting one. Animals thus seemed to have lost the positive reaction to stimuli, though not the negative one. No single place in the cortex could be found, the extirpation of which would lead to disappearance of all conditioned reflexes. On the basis of this it is possible to state that there is no such associative centre in the brain as supposed by Flechsig and

Demoor which would govern all associative activities. According to the works of the American school, it seems that in higher animals the localisation of the analysers is much more precise than in the lower animals, there being a large overlapping. Probably that is a reason why vicariation of function is much more developed in the lower animals than in dogs, or especially in man.

Experiments on Sleep

In describing internal inhibition it was mentioned that after several repetitions of an inhibitory stimulus there is a summation of the inhibitory after-effect. This inhibitory after-effect not only involves the analyser in which it originates primarily, but also spreads to the other analysers, with the consequence that all conditioned reflexes are weakened for a certain length of time. The effects of summation of the inhibitory after-effect can acquire such magnitude that all the conditioned reflexes may completely disappear. At the same time it appears that the inhibition spreads not only in the cortical areas, but also to the lower part of the central nervous system. Under these conditions the muscular tone at first slightly increases, then diminishes. The dogs no longer stand erect ; their heads droop, and they present the appearance of animals which are in a deep sleep. Repetition of positive conditioned stimuli does not lead to such results. Applications of strong extra stimuli may remove this condition of widespread inhibition, but only during the first few trials. The spread of internal inhibition may proceed at extremely rapid rates, so that on some occasions even a single application of a strong inhibitory stimulus may lead to immediate sleep. This is the case with long trace reflexes with a latent period of fifteen to thirty minutes ; after the application of the conditioned stimulus conditioned reflexes of other analysers may temporarily vanish, and the animal go to sleep for a time exactly equal to the latent period. As soon as the reflex is transformed

from the trace type into a simultaneous type all these inhibitory effects disappear, and the animal does not exhibit any effects due to inhibition. It appears that, from the point of view of conditioned reflexes, sleep can be regarded as an accumulation of internal inhibitions, which in this case is not concentrated within one analyser, but spreads over the whole cortex and the sub-cortical areas.

Temporary Instability of Conditioned Reflexes

Provided the general conditions of the animal do not alter, conditioned excitatory and inhibitory reflexes are very stable. Once a discrimination of two tones is reached, this discrimination will not alter provided no interruption is made in the work. At the same time it has been noticed that under certain conditions discrimination and the precision of trace and delayed reflexes diminish. This is especially so when new discriminations or fresh demands for internal inhibition are made. The following example will illustrate this. An animal is made to discriminate perfectly between a tone of 800 and one of 850 vibrations. Now a new discrimination between, say, two tactile stimuli is started, and it is found that while this is developing the older discrimination of the two tones becomes somewhat diminished in precision. Instead of a 100 per cent. discrimination the animal will give a discrimination of, say, 50 per cent. But as soon as the new discrimination has become firmly established the old discrimination returns to normal. The weakening of the older reflexes and inhibitions is a general phenomenon which is always observed during the process of establishment of new inhibitory activity. The finer the new inhibitory activity is, the greater will be its disturbing effect on previously established reflexes. Freshly established reflexes and inhibitions will in every case be more affected than older ones.

The injurious effect can sometimes be exaggerated to such an extent that we approach a state of the brain which is near to being pathological. Take the following example :

An animal is made to discriminate between a perfect circle and a very long oval. The discrimination is perfect. Now the oval is made broader. The animal at first does not discriminate the second oval from the circle, but soon establishes the new discrimination. Then wider and wider ovals are used, and in each case the discrimination is established, until we reach an oval which is, even to the human eye, very near in shape to a circle. The animal is now not only unable to establish the final discrimination, but in addition other conditioned reflexes and inhibitions become so upset that practically no normal conditioned reflex activity remains at all. Inhibitory stimuli become positive; positive stimuli become either negative or have a much weaker effect. The general behaviour changes too; the dog becomes very excitable, and has all the appearance of a neurotic patient. At this stage in the experiments an interval of a few months was made. The animal was then brought back to the laboratory, and it was found that most of the conditioned reflexes and inhibitions were again normal. The animal discriminated oval 1 from the circle, and after a few weeks ovals 2, 3, and 4. When oval 5 was reached the animal again showed this explosive change in its whole reflex activity. In the course of the work on conditioned reflexes there were met several other cases in which the same kind of change in the functions of the brain was observed, and in each case the reason was traced to the fact that exaggerated demands for internal inhibition had been made. It would appear that just as excessive accumulation of internal inhibitions leads to an inert state of the cerebral cortex and culminates in sleep, so a lack of adequate inhibition for a particular process leads to a state of general excitation.

Effect of Different Conditions of the Animal

It is imperative to stabilise the conditions of the experiments not only during the time of the actual provocation

of reflexes, but also during the time the animal is outside the experimental laboratory. Changes in the appetite of the animal and different states of hunger will affect the strength of conditioned reflexes. Digestive troubles, distemper, or an overfilling of the bladder or of the bowels, will first lead to a temporary disappearance or temporary weakening of all the inhibitory activities, and then even of the conditioned reflexes themselves. Pregnancy and inanition similarly affect conditioned reflexes, but for a longer period of time. It is extremely difficult to establish perfect discriminations or other inhibitory activities in an animal which is either pregnant or in a state of pronounced under-nutrition. A few days after delivery all conditioned reflexes will reappear.

Experiments were also performed on thyroidectomised animals, and these showed all the signs of cretinism. It was practically impossible to establish stable discriminations, and the development of all conditioned reflexes was slow.

Young animals normally rapidly establish conditioned reflexes as well as inhibitory reflexes; in old age these are more difficult, but it was always found that the conditioned reflexes which had been established during the younger periods persisted even to the last month of the animal's life.

Heredity of Conditioned Reflexes

Many efforts have been made to establish a definite proof of the hereditary transmission of acquired characters, particularly as regards the individual achievements of the central nervous system. Conditioned reflexes seem to afford a way of determining this question. So far the following experiments have been performed in this respect. Five puppies were removed from the mother and fed entirely on milk. At the same time a separate conditioned reflex was established with every puppy: with the first one to a sound of a metronome, with the second that of an induction coil, with the third the smell of camphor, with the fourth a continuous

red light, and with the fifth a rhythmic white light. Those stimuli were used as conditioned stimuli. The puppies were carefully preserved from any other food and from any contact with other animals. All conditioned reflexes developed quickly and were perfectly stable. After several months all the puppies were shown different objects, amongst them meat and other food substances which their ancestors were acquainted with. The puppies were prevented from smelling those foodstuffs. In no case was any kind of salivary activity observed. Meat, rubber corks, and matchboxes, were all alike to these animals. The same experiments were repeated with the smelling of different foodstuffs, and then both with showing and smelling. The result was the same : the animals did not exhibit conditioned salivary activity. The conclusion which must be drawn from these experiments is that carnivorous animals, which certainly from their very earliest periods of phylogenetic development have always had the opportunity of association of the sight and smell of food substances like meat, do not transmit those reflexes to the next generation. At the same time these experiments give an example of the conditioned reflex which is so general that every member of a species does develop it in the course of its life. And only by such experiments as we have just described was it possible to prove that the usual salivation of animals on sight and smell of meat is not really an inborn or unconditioned reflex, but an acquired one. The potentiality to acquire conditioned reflexes is a different question. It may be that heredity can transmit greater potentialities for the acquirement of such reflexes as had been acquired regularly by previous generations. Very recent experiments seem to have demonstrated that this may actually take place. Thus it was found in successive generations of mice that each generation acquired a particular conditioned reflex after fewer trials than did the preceding generation. The experiments, however, need further verification.

The Pharmacology of Conditioned Reflexes

The method of conditioned reflexes seems to be of great value for the determination of the action of drugs upon the highest parts of the central nervous system, for it enables us to experiment without the necessity of subjecting the animals to the detrimental effect of anæsthetics, electrical stimulation of nerves, or acute operative procedures. Only a few drugs have so far been tested.

It was found that *caffeine* augments all conditioned reflexes, and at the same time diminishes every inhibitory process, with the result that after a dose of caffeine of ·025 to 0·05 gram it is very difficult, or practically impossible, to extinguish a conditioned reflex. Discriminations vanish in delayed and trace reflexes; the secretion begins simultaneously with the application of the stimulus, so that the inhibitory pause during the latent period disappears. The effect of caffeine develops to a maximum in about twenty to thirty minutes after the injection, and lasts for at least one day.

Bromides, even in doses up to 1½ grams, do not affect the strength of conditioned reflexes. Their effect is mainly upon the inhibitory processes of the cortex, which in every case become very much strengthened. The inhibitory after-effect of an inhibitory stimulus becomes very prolonged, and spreads into the same and other analysers to a greater extent. The extinction of conditioned reflexes, which, as has been mentioned, is a special case of inhibited reflexes, is now obtained very easily, so that a reflex which takes ten to fifteen repetitions without accompaniment of the unconditioned stimulus to extinguish will now be extinguished after only two or three applications. Owing to their slow rate of excretion, the effect of bromides lasts for two to three days. The experiments with bromide show that, contrary to the usual teaching of pharmacology, this drug has no depressant action on the central nervous system, but

rather a specific reinforcing effect upon all its inhibitory activities.

Alcohol.—In agreement with most other pharmacological experiments, alcohol was found to be a purely depressant drug, small doses of alcohol affecting inhibitory processes, while larger doses diminished the strength of the conditioned reflexes themselves. *Strychnine* was also tried, and found to act very similarly to caffeine. The results obtained with some other drugs are not very conclusive, because by themselves they produce a spontaneous secretion of saliva. Other conditioned reflexes were, unfortunately, not tested.

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CHAPTER XIII

THE ACTIVE PRINCIPLES OF SOME ENDOCRINE ORGANS

Thyroxine—Pituitary Principles—Insulin—Parathyroid

1. Thyroxine

In 1914, a very potent crystalline compound was isolated from the thyroid by the brilliant investigations of Kendall, who gave to it the name thyroxine ; this substance contains 65 per cent. of iodine, and is no doubt the active principle to the presence of which the activity of iodothyrin was due.

Thyroxine is a colourless, odourless crystalline substance, melting at 231° C., insoluble in aqueous solutions of acids, soluble in caustic alkalies, to form salts. It also yield salts with acids.

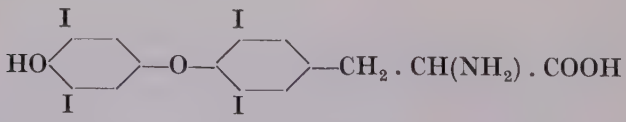
Harington has recently effected considerable improvements in the methods for the extraction of thyroxine, and thus obtained much larger yields than those given by Kendall's process. His method consists essentially in hydrolysis of the thyroid tissue with 10 per cent. baryta, precipitation with acid, and further hydrolysis of the acid-insoluble precipitate with 40 per cent. crystalline baryta, followed by separation of the thyroxine, first as the barium, then as the sodium salt, and finally in the free state. The yield was about 0·027 per cent. of the weight of the fresh gland, and the empirical formula of the pure substance was found to be $C_{15}H_{11}O_4NI_4$.

As a step towards the determination of its structure, Harington next removed the iodine from thyroxine by shaking an alkaline solution with a palladium calcium carbonate catalyst in a hydrogen atmosphere ; four atoms of iodine were

split off quantitatively, and four atoms of hydrogen took their place, the compound which was formed being called "*reduced thyroxine*" or "*thyronine.*"

The reduced thyroxine gave the ninhydrin and Millon's reactions, formed acid and basic salts, and contained its nitrogen in the form of an NH_2 group. Fusion with potash gave hydroquinone and *p*-hydroxybenzoic acid, which indicated the presence of two benzene rings; exhaustive methylation with methyl sulphate gave a betaine which split off trimethylamine to give an unsaturated acid containing one methoxy group, showing that there was one phenolic group in reduced thyroxine. The further oxidation products of the unsaturated acid suggested that reduced thyroxine might be a diphenyl ether of the structure

(viz., the *p*-hydroxyphenyl ether of tyrosine), and this was fully confirmed by the synthesis of the various degradation products and of this compound itself by two methods, both of which gave a substance identical with reduced thyroxine. Thyroxine has been shown by the further researches of Harington and Barger to be a tetra-iodo substituted derivative of this substance, which, by further analysis, they showed to have the structure



and this they finally confirmed by synthesising this substance, which had all the physical, chemical and physiological properties of the natural product.

Thyroxine is an extremely active substance, and a dose of 1 mg. produces definite effects on the basal metabolism of a normal individual. Daily doses of 2 mg. may raise the

basal metabolism of the normal individual 20 to 30 per cent. above the normal, but no effect is produced until about the fifth day after a dose has been given. It is believed that the human thyroid produces about 1 mg. of thyroxine per day (Plummer).

As extracted from the thyroid, thyroxine is always optically inactive, because it is racemised by the alkali used for hydrolysis of the tissue. Synthetic thyroxine is also, of course, inactive. Harington has recently resolved the synthetic form, and it has been shown by Gaddum that the *l*-thyroxine is much more active than the *d*-form. It may be presumed, therefore, that it exists as the *l*-form in the gland.

It is by no means certain that thyroxine is formed as such, or exists as such, in the thyroid ; most probably the iodine-containing and active principle is of the nature of a protein, of which thyroxine is one of the constituent amino-acids. The long delay which intervenes between the administration of thyroxine and the rise of basal metabolic rate may possibly be due to slow formation of the really active constituent, though it might equally well be due to other causes.

2. The Hormones of the Pituitary Body

In the pituitary body we have four important structures to be taken into consideration. These are the anterior lobe, posterior lobe, pars intermedia, and the pars tuberalis. It is not easy to dissociate their functions, or to say with precision exactly where the active constituents of the pituitary are formed. The anterior and posterior lobes and the pars intermedia have been known since the work of Herring, and need not be further described here.

The pars tuberalis was described by Herring as a part of the pars intermedia, but there is now good reason to regard it as histologically and developmentally distinct. Hogben and de Beer describe it as occurring in almost all animals. The pars tuberalis surrounds the infundibular stalk, and

spreads out on the base of the brain in the neighbourhood of the tuber cinereum. Atwell and Marinus describe it as histologically quite different from the pars intermedia and anterior lobe. It is much more vascular than the pars intermedia, and has an acinar structure; the eosinophile cells characteristic of the anterior lobe are wanting. Evidences of glandular activity, associated with the formation of a colloid material, have been described by Atwell. The interest of the pars tuberalis is due to the fact that it has recently been found that most of the results attributed to removal of the pituitary body can be obtained by merely injuring the hypothalamic region; these results are polyuria, glycosuria (?), loss of heat regulation, and early death. For further details the literature given in Bailey's article should be consulted. It is evident that the results of pituitary extirpation must be reviewed in the light of these facts.

Active Principles of the Anterior Lobe.—(1) *Growth-promoting Hormones.*—There is a good deal of clinical evidence in favour of the opinion that the anterior lobe of the pituitary body produces hormones which influence the growth of the skeleton. No definite hormone has been isolated, although in amphibian larvæ (*e.g.*, tadpoles or amblystoma) it has been shown by Allen, by Uhlenhuth and by Hogben, that administration of anterior lobe substance definitely accelerated metamorphosis and growth, while H. M. Evans, and also Hewitt, have recently succeeded in producing experimental gigantism in rats, by similar means. The nature of this anterior lobe hormone is still under investigation.

(2). *Hormone Affecting Reproductive Processes.* It has long been known that, in pituitary anterior lobe deficiencies, there is likely to be, in addition to a condition of dwarfism, an under-development and persisting state of infantilism of the generative organs, while, on the other hand, states of sexual precocity have also been associated with disorders of the pituitary body, possibly of a hypertrophic nature.

Definite experimental evidence in favour of the view that the anterior pituitary produces hormones which exert important effects on the reproductive organs was first brought to light in experiments of H. M. Evans and Long, of Smith, and of Zondek and Ascheim. Most of their work has been confirmed by Bellerby and extended by Parkes, to whose recent monograph the reader should refer for details.

This work shows that a hormone is produced by the anterior pituitary, which stimulates ovulation. When fresh pituitary suspensions are injected into adult females, an abnormally large number of ova ripen, and œstrus sets in. Ultimately, a large number of corpora lutea are formed, or, if pregnancy is established, an abnormally large number of embryos are discovered. This shows, then, that œstrus and ovulation are accelerated by the anterior pituitary hormones. The œstrus is not brought on in oöphorectomised animals, as it is by œstrin injections, and hence is due to a stimulant action of the hormone on the ovary.

When the macerated anterior lobe suspension is injected into immature mice, œstrus, with ovulation, occurs precociously, and even copulation may be allowed at the early age of nineteen days. Aqueous extracts having identical actions have been prepared by Zondek and Ascheim, and these workers have obtained what appears to be the same active principle from the urine of pregnant women, and have proposed a test for pregnancy, based on this. In this, the precocious development of œstrus, and the formation of multiple ripe follicles, corpora lutea or corpora hæmorrhagica, in young mice injected with the urine, are employed as the test. The principle is absent from male urine and from the urine of non-pregnant women.

There are indications that a second hormone may be present which is responsible for corpus luteum formation. At all events, sodium hydroxide extracts of the anterior lobe, which have been used by Evans and Long, cause extensive luteinisation in the adult, though not precocious œstrus in

immature animals. Zondek, however, considers that these differences are due to the large doses given by Evans and Long.

Further important evidence has recently been provided by the experiments of Fee and Parkes on rabbits. It is well known that the rabbit does not ovulate unless copulation has occurred; they showed that, if the anterior pituitary is removed a short time after copulation, then ovulation is withheld. This suggests that the normal process is that during coitus the anterior pituitary is stimulated; during the next few hours it liberates, in consequence, an increased amount of its hormone, and this induces in the ovary the changes associated with ovulation. If the pituitary is removed more than an hour after copulation, ovulation takes place normally.

Active Principles of the Posterior Lobe, Pars Intermedia and Pars Tuberalis.—Extracts made from the pituitary before and after removal of the anterior lobe were shown by Oliver and Sharpey-Schafer (whole pituitary) and Howell (posterior part) to cause a rise of blood pressure when injected intravenously, and this observation formed the starting point for the investigation of the active principles of the posterior lobe, with its attached parts.

The physiological effects which can be produced by injection of unpurified pituitary "posterior lobe" extracts ("pituitrin" and other trade names) are as follows:

(1) Rise of arterial blood pressure in the mammal (pressor action).

(2) Under certain circumstances a fall of arterial blood pressure in the mammal. This especially occurs after previous administration of pituitary.

(3) A fall of arterial blood pressure in birds (Noël Paton and Watson), and in reptiles and amphibia, either a fall or no effect (Hogben and Schlapp).

(4) Contraction of plain muscle, *e.g.*, of uterus of mammals, but no effect on that of invertebrates (Hogben and Hobson).

The production of contraction of plain muscle of the uterus is called the *oxytocic* action.

(5) Expansion of the melanophores (pigment cells) of the frog's skin (Hogben and Winton), so that the skin looks darker in colour.

(6) *Diuretic Effect.*—In anæsthetised animals a brief diuresis follows the injection.

(7) *Anti-diuretic Effect, checking an Existing Diuresis.*—This is seen clinically, as the administration of pituitary extracts subcutaneously or by intra-nasal insufflation checks diabetes insipidus.

It may easily be demonstrated on a normal subject: a litre of water is drunk, and the urine secreted each fifteen minutes is measured during the next few hours. Next day the draught is repeated, but immediately after it a small dose of pituitary extract is given subcutaneously. In a satisfactory experiment the diuresis is inhibited for several hours.

(8) *Galactagogue Effect.*—Administration of pituitary extract to a lactating animal leads to sudden expulsion of milk; this is no doubt due to contraction of the plain muscle in the walls of the milk sinuses.

(9) Effect on carbohydrate metabolism.

It now seems certain that there are at least two, possibly more, active substances in the extracts.

Herring believed that there were at least two different principles, and that the pressor substance was present only in the pars nervosa, which also possessed oxytocic properties, whereas the pars intermedia showed oxytocic properties, but had no pressor action. Hogben and de Beer have recently re-investigated this question, and though they found that extracts of the pars intermedia had definite pressor effects, yet on the whole they confirmed Herring's view that the extracts of the pars intermedia showed higher oxytocic than pressor activity, when compared with the extracts of the pars nervosa. Hogben and Winton have also shown that

extracts of pars intermedia are richer than extracts of the posterior lobe in the principle which affects the frog's melanophores.

Dreyer and Clark have found that it is possible by ultra-filtration through collodion membranes to separate the melanophore and uterine stimulants, the former being retained and the latter passing through. Further, they found indications that the vaso-constrictor and oxytocic substances were not identical. Knaus, Dreyer and Clark have by quantitative comparisons furnished additional evidence that the melanophore principle is distinct from the oxytocic and pressor principles.

Atwell and Marinus find that extracts of the pars tuberalis have but slight physiological activity, at all events as regards the pressor and oxytocic principles. Abel, on the other hand, believes that the pituitary hormone is present in this part.

Attempts at the Isolation of Pituitary Principles.—Among the earlier attempts to isolate an active principle from the pituitary extracts must be mentioned the work of Houssay and of Fühner. These workers obtained crystallised products ; the one isolated by Houssay in 1911 was thought by him to consist of a single substance. Fühner, collaborating with technical chemists, claimed to have isolated four crystalline sulphates, of which the first showed the pressor effects on blood pressure, the second the pressor and also the oxytocic activity ; the third was similar to the second, but had quantitatively a greater oxytocic effect,while the fourth product had a much greater oxytocic than pressor activity. The suggestion which springs from Fühner's work is that in all probability the pressor and oxytocic principles are distinct substances, which had been but incompletely separated by him. The difficulties of separation of the principles is no doubt due to the fact that they are all destroyed by similar treatment and all cling together on to any precipitates which are formed.

Dudley, and Dale and Dudley, after prolonged investigations came to somewhat similar conclusions. Dudley found that by extraction of the acid aqueous extract with butyl alcohol, a crystalline residue was obtained, which contained all the oxytocic substance, together with part of the pressor principle and some impurities. In further attempts to purify the oxytocic principle, he isolated a crystalline picrate of very great activity, but further study showed it to be potassium creatinine picrate contaminated with small amounts of the real pituitary principles, which must therefore be immensely active substances. From the mother liquors from the picrate, he obtained one fraction soluble and one insoluble, in acetone ; the insoluble portion had an activity, tested on the uterus, about twelve times that of histamine, since it acted in dilutions of one part in 1,250,000,000 ; this fraction also had a pure pressor effect on arterial pressure. The acetone-soluble fraction had a much smaller oxytocic action, and gave a fall of blood pressure, followed by moderate pressor effect. It seemed possible that there were four principles present, two in the acetone soluble, and two in the insoluble part. The evidence for this was that by extraction with suitable solvents, the effects could be partly dissociated.

The depressor principle which is present in small amounts in pituitary extracts appears to be a histamine-like substance ; Schafer and Vincent showed that this substance was extracted by alcohol, the residue then giving a pure pressor action only. The depressor effect which takes the place of the pressor response when pituitary extract is injected into etherised cats which have already received one or two previous injections of the same extract appears to be due to the presence of this alcohol-soluble substance, for Hogben and Schlapp, in repeating Schafer and Vincent's experiments, find that this depressor effect on repeated injection of the solution is lost when it has been evaporated and thoroughly extracted with alcohol beforehand. The depressor effect on the circulation

of birds, however, seems not to be due to the presence of the histamine-like substance, but is more likely to be due to the substance which, in mammals, produces the pressor response.

It is now generally agreed that commercial pituitary extracts contain small amounts of histamine, or histamine-like substances, to which the depressor action, and part of the oxytocic action, may be due. Dudley's work showed that the chief uterine stimulant was undoubtedly distinct from histamine; both are admittedly extracted from alkaline solutions by butyl alcohol, but from acid solutions the oxytocic principle is much the more readily extracted. Further, the pituitary oxytocic principle is rapidly destroyed by alkali, or by trypsin, while histamine is unaffected by either; lastly, histamine is soluble in boiling chloroform, in which the pituitary oxytocic principle is insoluble.

Abel and his collaborators held that the posterior lobe of the pituitary body contains only one specific principle. This substance they claimed to have isolated in an imperfectly purified form as a tartrate, which possesses all the properties of the pituitary extracts. In the course of purification of the substance, it is stated that the various properties of the pituitary extract are retained, and as concentration proceeds, the various effects augment together. The oxytocic power of this substance is at least 1,000 to 1,250 times as great as that of histamine, which is itself extremely powerful; the results of Abel's investigations thus agree with those of Dale and Dudley in showing that the pituitary principles are extremely active substances.

The controversy has recently been finally settled in favour of the multiple hormone theory, by the work of Kamm and collaborators, who have separated the pressor almost completely from the oxytocic principle. Their method consists briefly in extraction with dilute acetic acid, concentration of the extract at low temperatures, precipitation with ammonium sulphate, extraction of the precipitate

with glacial acetic acid, and fractional precipitation with ether, acetone and petroleum ether. Finally, two fractions were obtained, one predominantly pressor ("*vasopressin*"), the other predominantly oxytocic ("*oxytocin*"), and these have been prepared on the manufacturing scale. It is not yet certain whether the small pressor action of the oxytocic fraction is due to traces of the pressor principle, and *vice versa*, but Burn has shown that the anti-diuretic effect and the insulin-antagonising action are associated only with the vasopressin fraction. Gaddum finds, however, that this fraction has the melanophore-expanding action characteristic of extracts of pars intermedia, and hence is probably not a single principle.

Relation of the Pituitary Body to Diabetes Insipidus.—It is generally agreed that in the higher animals the posterior lobe of the pituitary can be removed without producing any symptoms at all, provided that no injury has been done to the pars tuberalis or contiguous structures. Camus and Roussy, and Bailey and Bremer, found that after removal of the posterior lobe, without effect, puncture of the hypothalamic region led to diabetes insipidus. Camus and Roussy explain this as due to injury to hypothalamic nerve centres, but Abel is of the opinion that the result is due to damage to the last remaining portions of pituitary structure capable of producing the anti-diuretic substance. Whatever the explanation may ultimately turn out to be, the results are of considerable interest in their connection with the clinical condition of diabetes insipidus.

That this condition is due to lack of an anti-diuretic hormone derived from the pituitary has, however, not been directly proved. Presumptive evidence has been provided by the experiments of Verney, who found that the polyuria of an isolated heart-lung-fed kidney was checked if the blood was circulated through a head, but not if the pituitary had first been removed from the head.

Brull and Eichholtz find that after injury to the tuber

cinereum the secretion of inorganic phosphates by the kidney is abolished.

Standardisation of Pituitary Extracts.—The hormones present in the usual mixed extracts are very powerful substances, and, in view of their extensive practical applications in medicine, it is important that there should be some reliable method for assaying their strength. For an account of the various methods the memorandum by Burn and Dale should be consulted. Their method consists in comparing the effect, on the isolated uterus of a 250-gram virgin guinea-pig, of the preparation under examination, with that of an extract made under definite conditions from standard dried powdered pituitary preparation ; the international unit of oxytocic activity is defined as the activity in 0·5 mgm. of the international standard powder. This tests the oxytocic power of the preparation ; since the pressor and oxytocic powers of preparations do not necessarily run parallel, it is desirable also to carry out tests to ascertain the strength of the preparation in pressor principle ; these tests are carried out on pithed cats. This method should, however, be used as a check on the uterus test, and not to replace it, since the practical purpose to which pituitary extracts are put is most often in connection with obstetrical work. Comparisons of four samples of commercial pituitary extract, by Bijlsma, Burn and Gaddum, gave oxytocic values of 7·1 to 13·7 units per cubic centimetre, pressor of 6·3 to 14 units and anti-diuretic of 20 to 24 units. The various activities did not run parallel.

3. Insulin

That removal of the pancreas produced diabetes mellitus was discovered by von Mering and Minkowski in 1889 ; while Schultze in 1900 proved that blockage of the duct led to atrophy of the gland, but not to diabetes. Hédon found that if a portion of pancreas was left in the body,

diabetes was prevented, although there was rapid sclerosis of the remaining gland substance. Sharpey-Schafer, in 1916, suggested that the maintenance of normal carbohydrate metabolism depended on the formation by the cells of the islets of Langerhans of a hormone, to which he gave the name of insulin.

Investigations by Allen, Lane, and Bensley gave further support to this hypothesis, and it had become generally recognised that the islets of Langerhans contained two types of cells, known as the α and β cells. Allen's work had indicated that it was the β cells which formed the internal secretion, and that diabetes mellitus was due to disturbance of function of these cells. Ligature of the pancreatic duct, or partial pancreatectomy, was not followed by diabetes, because the islet tissue did not atrophy as the acinar tissue did. But after partial pancreatectomy, when the amount of pancreas left behind was small, the animals (dogs) could be considered as on the threshold of diabetes; their carbohydrate tolerance was low, and if carbohydrate was given in excess of their tolerance, true diabetes often resulted. When this was the case, it was found that the β cells of the islets in the pancreas remnant had lost their granulation and become swollen. By careful dieting, with reduction of carbohydrate, it was sometimes possible to get the β cells to resume their functions, so that the animal returned to its former threshold state. Allen's system of diet for diabetic patients was based on this plan of allowing the islet cells as much rest as possible, and thus giving them every chance to recover their functions.

Several workers had partial success in the treatment of diabetes in man by the administration of extracts of pancreas, the most striking attempts being those of Zuelzer, in 1907, and of E. L. Scott, in 1911.

The workers who were most intimately concerned in the final successful attempt were F. G. Banting, C. H. Best, J. J. R. Macleod, and shortly afterwards J. B. Collip. They

were soon joined by other collaborators, too numerous for mention here, but to all of whom credit is due for some part of the knowledge which has since so rapidly accumulated.

Methods for Preparation of Insulin

Banting and Best started out with the idea that previous failures to secure the anti-diabetic substance in pancreas extracts might have been due to destruction of the hormone by the trypsin also present in the pancreas ; they therefore started with pancreas substance from dogs in which the pancreatic duct had been tied some time previously, and the glandular tissue allowed to degenerate. These extracts proved to be effective in reducing the hyperglycæmia and glycosuria of depancreatised dogs. They also found that extracts from fœtal pancreas, in which also there would not be a high concentration of enzymes, were capable of exerting an anti-diabetic action. Work was then directed to finding a method by which it might be hoped to obtain the hormone commercially from fresh adult pancreas. This object was achieved by the spring of 1922, and a depancreatised dog was kept alive for seventy days by administration of such extracts. Since that time there have been considerable improvements in the large scale methods for preparing insulin, and the products now on the market are quite reliable in their action, and accurately standardised as to their strengths. By the use of such preparations it is easy to keep completely depancreatised dogs alive and in normal health for indefinite periods, provided that enough fresh pancreas be also given by the mouth to supply the necessary digestive enzymes. Such dogs resemble severe human diabetics most closely.

Collip's method of preparing insulin consists in extraction of the pancreas with alcohol ; the extract is evaporated *in vacuo* to a small bulk, and the fat which separates is removed ; alcohol is then added to bring up the percentage

to about 80, and a precipitate of protein is separated ; more alcohol is finally added, and insulin is precipitated when the alcohol content is 92 per cent. Dudley and W. W. Starling improved this method by extracting with alcohol to which sodium bicarbonate has been added to render it slightly alkaline. The filtrate is acidified with acetic acid, and then treated much as in Collip's method. The yield of crude insulin is about five times as great as by the original method.

The crude insulin, however obtained, must be further purified. This may be most conveniently done by Dudley's method, in which it is precipitated from solution by picric acid, and the resulting insulin picrate then converted into a hydrochloride by treatment with alcoholic HCl, and finally with acetone, in which the hydrochloride is insoluble. The hydrochloride is filtered off and washed with acetone and then with ether, and dried. It forms a white amorphous powder readily soluble in water.

A simple method, which I have found suitable for students' use, is that described by Dickens and Dodds. It is a variation of a method used by Dudley. Fresh pancreas is minced with solid picric acid, extracted with acetone, and the solid picrate obtained by distilling off the acetone is then purified by Dudley's method.

The Origin of Insulin

It may be considered certain that the insulin of the pancreas is present in the islets of Langerhans. The granules of the β cells of the islets are soluble in dilute alcohol, in which the α cell granules are insoluble ; the β cells are the ones which suffer degenerative changes in diabetes.

In certain fishes (Lophius, Myoxocephalus, Gadus, Hippoglossus and Pleuronectidæ) the islet tissue forms a separate part of the pancreas, and it has been shown that insulin can be obtained in large amount from the islet tissue, but not from the other parts of the pancreas.

Substances which resemble insulin in their effects have also been prepared from clams, from yeast, and from plant tissues of the most varied description, by Collip. The insulin-like substance of vegetable origin has been called "glucokinin" by Collip, who claims that it differs in its action from insulin in acting more slowly, and for a longer time; and, further, that the blood of an animal rendered hypoglycæmic by glucokinin will, if injected into a second animal, render it hypoglycæmic too. These differences have been denied by others.

Various workers have been able to prepare from animal tissues other than the pancreas a substance which is apparently identical with insulin (Best, Smith and Scott).

The Blood-Sugar Level

The only sugar so far identified in normal blood is glucose, which is present in normal human blood in amount varying from 0·08 per cent. to 0·18 per cent., as an equilibrium mixture of the two stable forms called α- and β- glucose. The glucose in man is distributed between corpuscles and plasma, at all events after the blood has been shed.

A fact of the greatest interest is that the sugar content of the blood is not so constant as was at one time thought; it is true that the variations are not usually great, but they are nevertheless of the utmost importance. The chief cause of alteration of the blood sugar is the ingestion of carbohydrate food, for it is now known that the liver does not at once remove the whole of the absorbed carbohydrate which is passed to it in the portal circulation.

The effects of ingestion of carbohydrate are best seen in the determination of what is known as the *blood-sugar curve*. For this purpose the fasting level of the blood sugar is determined, and then 50 or 100 grams of glucose dissolved in water are taken by the mouth. Samples of blood are drawn for sugar estimation each half hour for three hours. In most normal people the fasting blood-sugar level is about

0·08 per cent.; within thirty minutes after taking glucose this has risen to about 0·18; in one hour the amount has begun to fall, and at the end of one and a half to two hours after the glucose meal the blood sugar has returned to normal again, or may even be a little below. If insoluble carbohydrates, such as starch, be taken instead of glucose, the rise is often smaller, and of slightly longer duration, owing to the relatively slow digestion and absorption of the products.

Another condition in which the blood sugar shows definite change is after parturition, when it may fall even to a dangerous level owing to withdrawal of glucose by the mammary glands for formation of lactose. The "milk fever" sometimes seen in cows after calving is apparently due to this, and may be relieved by injection of glucose, or by inflation of the udders with air to check the circulation in the mammary glands.

Glycosuria.—The urine of normal persons always contains traces of a reducing substance, corresponding to 0·03 to 0·1 per cent. of glucose, though the reducing substances have not been clearly shown to be glucose; this amount of reduction cannot be detected by the use of the usual reagents; when the urine contains 0·2 per cent. of glucose and upwards, it certainly reduces Benedict's solution, and the condition is then termed glycosuria.

The occurrence of glycosuria depends upon two things: (1) The sugar content of the blood; (2) the renal sugar threshold. When glycosuria occurs it means that the blood sugar has risen to a value higher than the renal threshold; consequently, glycosurias may be due to one of two classes of cause; first to an abnormally high blood sugar, secondly, to an abnormally low renal sugar threshold. The normal renal sugar threshold is 0·18 per cent., that is to say, glucose will not be excreted, or not in such amounts as to be readily detectable, unless the blood sugar rises above 0·18 per cent. In some rare individuals the renal threshold is low,

say 0·1 to 0·12 per cent., and this means that sugar will readily leak away when a glucose test meal is given, even though the blood sugar may not rise so high as it would in a normal subject. This condition is called *renal diabetes ;* according to Graham the prognosis is not a serious one.

The normal individual can take very large amounts of glucose (several hundred grams) without losing more than traces of glucose in the urine ; his *sugar tolerance* is high.

Of the various causes of glycosuria, the most interesting in the present connection is that of diabetes mellitus. When the subject of this disorder is given a glucose tolerance test, combined with a blood-sugar curve, it is found that : (1) in serious cases the fasting blood-sugar level is high (0·2 to 0·4 per cent.) ; (2) the rise of blood sugar after ingestion of the glucose is greater than would be found in a normal person, and is of longer duration ; (3) the glucose tolerance is low, that is to say, glucose is excreted in considerable amount in the urine, even when relatively small amounts have been taken, or in definitely established cases, even when no carbohydrate has been ingested at all. The hyperglycæmia which results in animals from removal of the pancreas is similar in every way to that due to diabetes mellitus in man.

Other causes of glycosuria need be only briefly referred to here, as they are usually discussed at some length in the textbooks. Some of the varieties are as follows :—

(1) Adrenaline glycosuria, to which may be added glycosuria associated with discharge of adrenaline from the suprarenals, *e.g.*, piqûre, asphyxia, stimulation of splanchnics, administration of anæsthetics or morphine.

(2) Stimulation of the nerves to the liver.

(3) Phloridzin glycosuria ; this probably acts by antagonising the influence of insulin and also exercises an incidental effect by lowering the kidney threshold. The effects of phloridzin poisoning present many similarities to pancreatic diabetes.

The first two of these types of glycosuria (but not phloridzin

glycosuria) are accompanied by *hyperglycæmia*, or raised blood sugar, due to accelerated breakdown of liver glycogen.

Effects of Insulin

General Effects.—Insulin is given by hypodermic injection of the solution ; it is ineffective if given by the mouth ; most of the experimental work has been carried out on rabbits, cats and dogs. The blood-sugar level falls rapidly for the first thirty minutes ; in animals previously starved for twenty-four hours, and given relatively large doses of insulin, the blood sugar falls to 0·04 per cent. or even lower, and then there follow very curious effects of this *hypoglycæmia*. Rabbits show signs of extreme hunger as the blood sugar falls, and will eat paper or shavings with readiness ; when the blood sugar has fallen to 0·045 per cent., a certain proportion of the animals suddenly become seized with violent convulsions, resembling those due to asphyxia ; between these seizures the animal is comatose, with rapid, shallow breathing. After a series of convulsive attacks, each lasting for half to one minute, with alternating periods of coma, the convulsions become feebler, the body temperature falls, and soon the animal dies of respiratory failure. *Rigor mortis* sets in at once.

Dogs become restless, and have tachypnœa, foam at the mouth and bark violently, the sphincters are relaxed, and convulsions set in, terminating as in the rabbit. Mice at ordinary temperatures merely show signs of weakness, with fall of temperature, but Krogh has found that if kept in a chamber at about 28° they also have convulsions.

In man also, symptoms of overdosage of insulin have been witnessed, both in experiments and in the course of treatment. The first symptom is hunger pains ; then follows a feeling of fatigue, faintness, tremor and anxiety, sometimes with loss of emotional control or even temporary delirium ; pallor or flushing and profuse sweating are usually seen, and in extreme cases there is deep coma.

All these symptoms can be removed in a few minutes by the timely administration, by the mouth, or if necessary intravenously, of glucose in suitable amount; in any case, the symptoms of hypoglycæmia are not likely to be seen except when the animal or person is fasting, and the dose of insulin is large. In the treatment of diabetics, therefore, the administration of insulin should be accompanied by the ingestion of a sufficient amount of carbohydrate to obviate the occurrence of these symptoms.

The convulsive effects of excess of insulin appear to be due to an effect of the lowered blood sugar on the nerve centres of the pons and medulla; it is not understood exactly in what way a lowering of blood sugar brings about such results, but it has been suggested that there is some abnormality in the oxygen usage of the nerve cells which are affected.

The Effect of Insulin on the Blood Sugar.—The effect of insulin in lowering the blood sugar is seen not only when the sugar level is normal to begin with, but also when, from any cause, the blood sugar has been previously raised. Thus, if animals are rendered hyperglycæmic by piqûre, adrenaline, asphyxia, ether or morphine, glucose injection, or by pancreatectomy, the blood sugar is quickly lowered again by administration of suitable amounts of insulin. The same applies to the hyperglycæmia of diabetes mellitus in man.

One of the first questions which arose from the work on insulin was the fate of the blood sugar which so rapidly disappeared when insulin had been given. In attempting to answer this question we must first of all consider what are the sources of the tissue carbohydrate and blood sugar in both the normal and the diabetic individual. In the normal animal they may be regarded as derived either directly from ingested carbohydrate, or indirectly from the breakdown of protein, and probably to some extent also from fat. One thing seems certain, that, whether carbohydrate is ingested or not, its presence and continual metabolism in the tissues is essential to life and probably represents the immediate source

of the energy liberation which is correlated with all life-processes. There is good reason to suppose that when carbohydrate is not supplied to the body as such there is production of carbohydrate in the body from fat, and we also have indisputable evidence of the conversion into carbohydrate of the deaminised residues from protein breakdown. The amount of carbohydrate at the disposal of the body at any time thus depends upon (1) the store present as glucose and glycogen in the blood and tissues; (2) the amount accruing from absorption of carbohydrate; (3) the amount formed from protein and fat.

Conversion of protein and of fat into carbohydrate is effected, not in the tissues as a whole, but in the liver; hence, when the intact animal is studied it is found to have a respiratory quotient which, in accordance with the requirements of such a transformation, falls below unity by an amount proportional to the extent to which the animal is being supplied with carbohydrate of endogenous origin. The eviscerated animal, deprived of its liver, on the other hand, has, according to Burn and Dale (see Table, p. 429), always a respiratory quotient of 1·0, and its metabolism is therefore confined exclusively to carbohydrate.

A similar indication of the relation which the liver bears to endogenous carbohydrate formation is provided by the experiments of Mann and his collaborators on liver extirpation. They found that unless the diet contained an adequate amount of carbohydrate, the blood sugar of these animals rapidly fell to a convulsive level; the convulsions could be stopped by intravenous injections of glucose.

The amino acids derived from protein breakdown, and the fats, can both give rise to ketone bodies as well as to sugar. The proportion of an amino acid convertible into ketone bodies varies with each particular amino acid, but under suitable conditions it appears that about 58 per cent. of the weight of protein and 10 per cent. of the weight of fat taken as food are convertible into glucose, while not more than

about 46 per cent. of the protein but 90 per cent. of the fat are capable of conversion into ketone bodies. Both fats and proteins are thus *ketogenic*. Since the combustion of carbohydrate is essential for the proper oxidation of the ketone bodies, the sugar or sugar-yielding parts of the diet are sometimes spoken of as *anti-ketogenic*. Whatever the theoretical explanation may be, it is an undoubted practical fact that if the ratio of anti-ketogenic to ketogenic substances be not sufficiently large, ketone bodies escape oxidation and enter the blood, to be excreted in the urine and breath—a condition called ketosis ; ketosis is thus seen in starvation, or in feeding with a diet too rich in fat.

The metabolism of the diabetic animal is characterised by partial failure of the liver and muscles to store carbohydrate, and further by the *accelerated endogenous production of carbohydrate* from protein, and probably also from fat. The basal metabolism is considerably increased, no doubt largely for this reason. It has often been stated that the tissues also exhibit a diminished power to burn glucose, and though this may be conceded, the power of oxidising sugar is certainly not abolished. With increased production and diminished storage of carbohydrate the blood is flooded with sugar, which escapes by the kidneys, while the accelerated breakdown of protein and fat leads to the formation of ketone bodies in large amount.

In the diabetic subject, it seems quite clear that the effect of administering suitable amounts of insulin is to restore the carbohydrate metabolism to normal ; in other words, to accelerate the combustion of carbohydrate and to allow the liver and muscles to carry a store of glycogen ; hence, to lower the blood sugar ; because the carbohydrate metabolism is restored, and because new formation of carbohydrate is reduced, ketone bodies speedily disappear from the blood, and therefore from the urine and breath. It is evident that, in the diabetic, insulin produces all these effects by supplementing the inadequate action of the pancreas.

In the normal animal or man, there is already an efficiently functioning pancreas, so that in administering insulin to them we are getting abnormal effects due to the presence of an excess of that substance in the body. The effects of this excess of insulin are best understood if we consider them as an exaggeration of the effects of therapeutic doses on the diabetic, *i.e.*, as displaying the same qualitative influences as in the normal metabolism of carbohydrate, but to an enhanced degree.

We should therefore expect the following effects to ensue when insulin is given in rather large doses to the normal animal; first, an increased storage of glycogen in the muscles and liver, together with an accelerated combustion of glucose in the tissues. Secondly, a reduction to a level far below the normal of the formation of carbohydrate from protein and from fat. In consequence of both of these effects, there would be a large reduction in the blood-sugar level, with its attendant consequences. One of these consequences is the onset of convulsions; another is that the liver glycogen is now drawn upon, as will be explained later. How long the liver and muscles will be able to supply carbohydrate to the animal which is now deprived of the normal endogenous new formation of sugar will depend on the total available store of carbohydrate in the body at the time the dose of insulin was given, but it is certain that in fasting animals with rapid metabolism, such as mice, the store is very small, viz., about 170 mg. per 100 grams of body weight. If the animal were oxidising carbohydrate directly and exclusively for its metabolism, however, its oxygen consumption shows that it would require about 560 mg. of carbohydrate per 100 grams per hour, and therefore its store would last for only about twenty minutes if new formation from protein and fat ceased. If the action of insulin is that explained above, we should expect to find that, by stopping new formation and thus confining the animal's metabolism to its ready-formed carbohydrate, there would, if no more carbo-

hydrate were supplied, be a rapid falling off in the respiratory exchange, accompanied by a fall of body temperature, while if the fall of body temperature were prevented by placing the animal in a thermostat, we should expect that the animal would die in about half an hour, or as soon as its carbohydrate was completely exhausted. This is what actually happens, and in other species of animals the fatal result depends on similar conditions, and is accompanied by a similar depletion of the body store of carbohydrate.

In the later stages, therefore, the glycogen content of the liver and muscles, instead of being raised, is greatly lowered.

The account here given of the actions of insulin is the one which appears to the author to be the simplest and most comprehensive. In several respects, however, the whole question is actually far less comprehensible than this description may lead the reader to suppose, and there is almost no statement in this account that might not be denied or greatly qualified by one or more of the hundreds of investigators who have approached the problem. For instance, Goldblatt has shown that in young starving rabbits, insulin causes considerable increase in the liver glycogen, even when hypoglycæmic convulsions occur and cause death. This finding is directly opposed to the view that insulin suppresses gluconeogenesis, and in fact Goldblatt believes that new formation of glycogen from endogenous sources is increased by insulin. Other experimenters, working under different conditions, seem to have obtained equally clear evidence that insulin reduces gluconeogenesis, as argued above.

The emptying of the liver glycogen in consequence of the fall of blood sugar is due to the effects of the lowered blood sugar on the central nervous system, from which efferent impulses pass to the liver *viâ* the sympathetic nerve supply. Hence, when these nerves are divided or paralysed by ergotamine, it was found by Burn that the fall of blood sugar produced by insulin is much greater and lasts much longer than under ordinary circumstances.

We will now consider the fate of the glucose which disappears from the blood under the influence of insulin : some of it is burned, and the rest disappears into the tissues.

It has been shown that glucose is not destroyed by glyco-

lysis at a greater rate by blood to which insulin was added than by blood alone, but that when circulated through a heart, or through a decapitated cat preparation consisting only of heart, lungs and muscle, glucose disappeared from the blood at a greater rate when insulin was added than when it was absent. In experiments of this nature by Burn and Dale, there was a definite normal rate of utilisation by the tissues, which, in the absence of the liver, led to a rapid reduction of the sugar content of the blood : in order to make this good they gave slow infusions of glucose at such a rate as to keep the blood sugar constant, or slowly rising. When now insulin was added to the circulating blood, the blood-sugar level rapidly diminished in spite of the continued infusion. By accelerating the infusion rate, the fall could be prevented, and it was found that in order to accomplish this the rate of infusion had to be increased many times. There was in these experiments an increased oxygen usage after the insulin, but, as shown in the table

Effect of Insulin on Decapitated Eviscerated Cat
(Burn and Dale.)

Before Insulin.				After Insulin.			
c.c. O_2 per hour.	R.Q.	Glucose used, mg. per hour.	c.c. O_2 needed to burn glucose.	c.c. O_2 per hour.	R.Q.	Glucose used, mg. per hour.	c.c. O_2 needed to burn glucose.
465	1·02	351	262	489	0·92	783	585
594	1·06	—	—	622	0·99	996	744
441	0·99	444	332	498	1·01	1,021	762

above, this increase is quite inadequate to explain the loss of much of the carbohydrate by combustion.

In continuation of these investigations it was shown by Best that insulin also accelerates the glucose disappearance from blood perfused through an isolated limb.

It seems certain from these and other investigations which cannot be detailed here, that the glucose, or at least much of it, suffers some non-oxidative change, and is withdrawn from the blood to enter the tissues, particularly the muscles. A suggestion was made by Lesser to the effect that part of the glucose was oxidised, and, as happens in the oxidation of lactic acid in muscle during recovery, the remainder of the glucose was built up into glycogen, and researches by Best, Dale, Hoet and Marks have given good grounds for believing that the explanation lies along some such lines.

Best, Hoet and Marks showed that when the blood sugar of the eviscerated spinal cat was kept at a high level for two to five hours by intravenous injection of glucose there was no demonstrable change in the glycogen content of the skeletal muscles, when the muscles of one side were compared with the corresponding muscles of the opposite side of the body. When insulin was also given, so as to produce an accelerated rate of sugar disappearance, there was an *increase* in the glycogen content of the muscles, irrespective of whether actual hypoglycæmia resulted or not, and this deposition of the glycogen was enough to account for from 40 to 50 per cent. of the sugar which had been lost from the blood. It may be concluded, then, that under the conditions of these experiments, much of the lost sugar was recoverable as glycogen. In the spinal animal convulsions do not occur, and the depletion of muscle glycogen of normal rabbits after insulin must be due to the muscular activity associated with the convulsions.

Best, Dale, Hoet and Marks showed that the sugar which disappears from the body can be completely accounted for by combustion on the one hand and conversion into glycogen on the other hand. In their experiments is given a complete balance sheet of carbohydrate in an eviscerated spinal cat, before and after insulin administration. In these experiments the liver was not removed, but it gave up some glucose by diffusion into the hepatic veins, and this had to

be allowed for in the results. Glucose was infused continuously, and there was continuous determination of the oxygen usage. Samples of liver and muscles were taken at the start and finish for determination of glycogen and free glucose, and blood samples were also taken at intervals.

The following balance sheet was constructed :—

SOURCE OF SUGAR

(1) Liver carbohydrates before insulin (as glucose)	= 3·045 per cent.
Liver carbohydrates after insulin period	= 2·195 ,,
Loss of glucose from liver	= 0·85 ,,
Weight of liver = 102·6 grams, *i.e.*, total loss	= **0·872** grams — — glucose
(2) Free muscle sugar before insulin	= 0·320 per cent.
Free muscle sugar after insulin	= 0·240 ,,
Loss of free glucose from muscles	= 0·080 ,,
Weight of muscle = 1,400 grams, *i.e.*, loss	= **1·12** grams. — —
(3) Total weight of sugar infused	= **1·68** ,, — —
(4) Blood sugar fell from 0·360 to 0·034 per cent.	= 0·326 per cent.
Volume of blood = 175 c.c., *i.e.*, total loss	= **0·570** grams. — —
Total sugar to be accounted for	= **4·242** ,,

FATE OF SUGAR

(1) Muscle glycogen before insulin (as glucose)	= 0·727 per cent.
Muscle glycogen after insulin (as glucose)	= 0·799
Increase	= 0·072
	i.e. = **1·008** grams.
(2) Oxygen used during experiment = 2,369 c.c. at N.T.P. = 2,369 × 4/3 mg. glucose burnt	= **3·079** grams. — —
Total sugar accounted for	= **4·087** ,,

This agreement between the sugar lost and that accounted for as either burnt or deposited as glycogen is as good as

could possibly be expected in view of the elaborate nature of the experiment and of the calculations involved.

In this particular experiment there was only a limited deposition of glycogen, as might be expected from the small amount of sugar available. In other experiments, in which larger quantities of glucose were offered, the glycogen deposition was much greater; thus in one instance in which 7·28 grams of glucose were infused, the average glycogen content of three muscles of the right side before insulin was 0·45 per cent., and that of the corresponding three muscles of the opposite side five and a half hours after the administration of insulin was 0·81 per cent.

The Normal Function of Insulin

Large amounts of carbohydrate can be dealt with by the normal individual without any appreciable loss of sugar by way of the urine; on the other hand, abstinence from carbohydrates for quite long periods does not lead to any definite symptoms of hypoglycæmia. This is no doubt due to the fact that protein, and to a less extent fat, can be converted into glucose, and thus supply the imperative needs of the body for carbohydrate. But all the experience of clinicians in the treatment of diabetes by insulin has shown that it is necessary to secure a proper balance between the amounts of insulin administered and carbohydrate ingested, as otherwise there will be either an excretion of glucose in the urine, or else the unpleasant effects of hypoglycæmia. In the normal functioning of the pancreas, since neither of these effects ensues, there must therefore be some means by which the amount of insulin liberated from the islet tissue corresponds with the amount of carbohydrate to be dealt with.

Apparently, when carbohydrate is taken by the mouth, as in the administration of glucose for the purposes of a test meal and blood-sugar curve, there is an accompanying liberation of insulin to deal with it; it is often seen that in the construction of a blood-sugar curve the blood sugar falls very

rapidly after its initial rise, and reaches a level slightly below that of the blood in the fasting state. The rapid fall is not because the absorption of the glucose from the alimentary canal is at an end; this is shown by the fact that in the diabetic subject the rapid fall is not seen, the high level persisting even for some hours after the ingestion of glucose which originated it. The fall in the normal subject without doubt occurs because by that time the insulin production has been sufficient to reduce the blood sugar in spite of the continuing sugar absorption. For this reason it is found that if a second test meal of 50 grams of glucose is given to a normal subject as soon as the blood sugar has reached its low level, there is little or no further rise in the blood sugar. During fasting the sugar contents of the arterial and venous bloods are practically identical, according to Lawrence, but after taking glucose the venous blood sugar may be 0·02 to 0·05 per cent. lower than the arterial. In diabetics this difference is not seen, except when insulin is given with the carbohydrate. Here again we have evidence of the liberation of insulin in the normal and of its lack in the diabetic subject. It has even been shown that a transfusion into a diabetic subject of blood taken from a normal individual during the absorption of glucose will lead to a fall in the blood sugar of the patient, just as though insulin had been given. Macleod and collaborators have given some evidence which supports the view that the liberation of insulin is controlled by influences reaching the pancreas by way of the vagus. Whether this effect is produced by the action of hyperglycæmic blood on the nerve centres, or whether the pancreas also reacts directly to a rise of blood sugar, which seems likely, are questions that cannot yet be answered.

Relation of the Pancreatic Islets to other Endocrine Organs.—Just as the hyperglycæmia produced by adrenaline can be counteracted by insulin, so the hypoglycæmia brought about by an overdose of insulin can be relieved by admini-

stration of adrenaline if any glycogen is present in the liver.

Cannon, McIver and Bliss have shown that when insulin is given there is excitation of the sympathetic system and a discharge of adrenaline from the suprarenals as soon as the blood sugar falls below about 0·07 per cent., which checks the further fall of blood sugar; conversely it is likely that the injection of adrenaline into the circulation leads to a setting free of insulin from the pancreas.

That the internal secretion of the thyroid exerts an effect on carbohydrate metabolism is an old claim, which has recently been investigated by Burn and Marks, with most interesting results. They find, as did Cramer and Krause, that feeding with thyroid reduces the glycogen store of the liver, and makes the liver glycogen more readily available on call. Hence, provided there still remains some glycogen in the liver, injection of adrenaline calls forth a greater hyperglycæmia than usual, while (no doubt correlated with this) administration of insulin gives a much smaller fall in blood sugar than would be expected. But when, as a result of thyroid feeding, the liver has been quite depleted of glycogen, then, for obvious reasons, adrenaline gives a smaller hyperglycæmia than previously, while the effect of insulin is greatly enhanced, so that very small doses may now cause a fall of blood sugar to the convulsive level. In one instance they even found that administration of a small dose of glucose led to death of the animal with hypoglycæmic convulsions; the explanation was that the adrenaline which was turned out in response to the increased formation of insulin was unable to generate any sugar from the depleted liver, so that the insulin produced by the animal's own pancreas in response to the dose of glucose proved too much. Removal of the thyroid was found by Burn and Marks to have the opposite effect to moderate feeding with thyroid, namely, to increase the sensitiveness of the animal to insulin, while reducing the intensity of the hyperglycæmic response to adrenaline.

Standardisation of Insulin.—Typical insulin should possess the following properties :—

(1) It should cause immediate reduction in the blood sugar of fasting rabbits, and when convulsions ensue these should be at once removed by intravenous injection of glucose.

(2) When given to diabetic (depancreatised) animals all the symptoms of diabetes should at once disappear, and if carbohydrate is given the respiratory quotient should rise, and glycogen should be deposited in the liver.

The strength of insulin preparations must be carefully controlled for use by biological standardisation. Various methods, mostly based on the reduction of the blood-sugar level of animals, have been tried for this purpose. At one time a unit of insulin was defined as the amount which would in four hours lower the blood sugar level of a 2 kg. rabbit to the convulsion stage (0·045 per cent.). This unit, the original Toronto unit, was found too large, and the international unit now used is about one-third of this. The present method is to determine the fall of blood sugar brought about in five hours in a fasting rabbit, as compared with that induced by an international standard preparation, of which $\frac{1}{8}$ mg. contains 1 unit. Varying with the amount of carbohydrate given, each clinical unit of insulin can bring about the utilisation of varying amounts of sugar, the glucose equivalent of insulin becoming smaller with increasing dose.

The Chemical Properties of Insulin.—The chemical nature of insulin is at present a matter for speculation. The substance is stable in aqueous solutions of acid or neutral reaction, even at 100° C., but in alkaline solution is rapidly destroyed, even at body temperatures. It is also inactivated in alcoholic solutions containing HCl, but its activity is restored again by treatment with dilute alkali in the cold (Carr, Underhill, *et al.*). Similar inactivation, reversible by alkali, occurs by treatment with acetic anhydride. It is probable that the inactivation, in both instances, is due to esterification, which would indicate the presence of free

carboxyl groups in the insulin molecule. Insulin is also destroyed by trypsin, and by pepsin even in acid solutions ; it often gives certain of the protein colour tests, *e.g.*, Millon's and the glyoxylic reaction, but these are said to disappear when it is purified ; it has been stated that when highly pure not even the biuret test is given. Purified insulin also gives the ninhydrin reaction. That it contains an iminazole ring compound is shown by the fact that it gives the Pauli colour reaction ; it also contains sulphur, the content of which increases as the purification proceeds. Abel and Geiling have shown that the sulphur is readily removable with hot dilute sodium carbonate, and that the amount of labile sulphur is proportional to the activity of the sample of insulin. Abel, in 1926, claimed to have isolated a crystalline insulin, and Harington and Scott, by a somewhat different method, have confirmed this. Their method consists essentially in carrying out isoelectric precipitation in presence of saponin. The crystals were of uniform properties, composition and physiological effects. Analysis showed 3·11 per cent. of sulphur. Independent determination of various samples by different observers gave a physiological potency of 22·7 to 23·9 units per mg., or an average of $23{\cdot}3 \pm 0{\cdot}6$. It is thus about three times as potent as the international standard. Many commercial samples of insulin reach a potency of 20 units per mg. There are many indications that insulin is a protein substance, and if so, two of the most likely component amino acids are histidine and cysteine. Insulin is carried down with many precipitates, because it is strongly adsorbed to them, and various methods of purification are based on this fact, which incidentally happens to be one of the chief obstacles to its final isolation.

4. The Parathyroid Hormone

It has long been known that after removal of the parathyroids tetany is produced, which terminates in death in

a few days. This condition of tetany is associated with increased excitability of the central and peripheral motor nerve system, is usually associated with a low calcium content of the blood, and can temporarily be relieved by administration of calcium salts.

There are two principal theories put forward to account for these symptoms. Noel Paton and his colleagues were of the opinion that the condition is due to the accumulation in the body of guanidine or its derivatives, the removal of which is normally controlled by the parathyroids. It is certain that parathyroid tetany can be closely imitated by the injection of guanidine or methyl guanidine into the circulation, and the disturbance so produced resembles parathyroid tetany by being amenable to the administration of calcium salts. Howland and Marriott have found that the normal blood calcium in human beings is of the order of 10 mg. per 100 c.c. of serum, whereas the serum calcium of patients suffering from idiopathic tetany is of the order of 5 or 6 mg. per 100 c.c. The second theory is related to these facts and regards the parathyroids as responsible for producing a hormone regulating the blood calcium level. According to this theory tetany is due directly to reduction of the blood calcium. Greenwald has given reasons against the belief that there is a toxic substance in the blood of parathyroidectomised animals and has shown that the only striking changes following parathyroidectomy are the lowering of blood calcium and a diminished excretion of phosphorus in the urine.

The parathyroid tetany is modified by various conditions, some of which act in a manner not understood. Thus, it is abolished or reduced by intravenous injection of calcium-free saline solutions, by bleeding, and by diets rich in lactose; further, after the early stages, parathyroidectomised animals often become habituated and remain free from tetany; this may perhaps be explained as due to hypertrophy of parathyroid remnants.

Pregnancy, lactation, or meat diet accentuates the tetany. Although pregnancy and lactation in normal animals are not accompanied by reduction in the blood calcium, both conditions undoubtedly put a strain on the calcium reserves of the body.

Removal of the colon has been shown to reduce the tetany, and it is conceivable that this may be related to the fact that calcium salts are normally excreted by way of the large intestine.

Active Principle of the Parathyroid.—Evidence which weighs heavily in favour of the calcium control theory has been produced in recent years by Collip, who has been able to prepare extracts of the parathyroid capable of keeping dogs in normal health after parathyroid extirpation, and therefore presumably containing the active principle. The method of preparation of these extracts is briefly as follows :—

The fresh glands are heated on the water bath with an equal volume of 5 per cent. HCl for one hour. The oily layer of fat is removed and the extract cooled and brought to *p*H 8 by addition of sodium hydroxide. Hydrochloric acid is then slowly added until maximal precipitation of protein occurs. This precipitate is removed and may be re-extracted. The filtrate is subjected to further purification by being made acid to Congo Red and then saturated with sodium chloride. The precipitate so formed is dissolved in water containing dilute HCl and again reprecipitated by sodium chloride. This reprecipitation may be repeated several times, the precipitate finally being dissolved in weak alkali, which then has its *p*H adjusted to 4·8, at which reaction an isoelectric precipitate forms ; this is removed, redissolved, and the isoelectric precipitation repeated. Finally, the precipitate is dissolved in weak acid at *p*H 4, or may be obtained as a powder by suspending in absolute alcohol and adding an equal volume of ether. The resulting precipitate is washed in ether and dried over sulphuric acid.

When the solution of this extract is administered orally or parenterally to normal or parathyroidectomised dogs, there is a rise in the blood calcium, and parathyroidectomised dogs or patients may be cured of their tetany by periodic administration of the extract. The potency of the extract is determined by administration to strictly dieted dogs, and the increase in blood serum calcium produced in definite time is determined. The unit of parathyroid extract has been defined by Collip as 1/100th of the amount required to produce a 5 mg. per cent. rise in blood serum calcium in fifteen hours in a dog of 20 kilos weight; his purest dry preparation contains 1 unit per 0·4 mg.

When repeatedly administered to normal dogs in large amounts considerable rise in blood calcium results, with ultimate disastrous consequences to the animal. These symptoms set in when the serum calcium rises above 15 mg. per cent., and consists of sanguineous vomiting, weakness, diarrhœa, and finally collapse and death. As soon as the calcium rises above the normal there is also an increase in blood phosphorus, which rises to very high levels shortly before death; there is also a premortal rise in the non-protein nitrogen of the blood. In the parathyroidectomised animal blood calcium rises at suitable dosage to its normal level. It seems probable that the hormone produces mobilisation, both of calcium and of phosphorus, from the bones. Greenwald and Gross are of the opinion that the parathyroid hormone enables calcium phosphate to remain in solution in the blood and that calcium phosphate is thrown out from the blood when the hormone is absent in consequence of parathyroidectomy. It has been directly shown by Stewart and Percival by filtration of the blood through collodion that administration of parathyroid extract increases the diffusible blood calcium from about 65 per cent. of the total blood calcium up to about 85 per cent. Collip has also shown that the symptoms of over-dosage with parathyroid can be imitated by administration of calcium

chloride and mono-sodium phosphate together, though not by either alone.

The chemical nature of the parathyroid hormone is unknown. Collip and Clark found their purest material to contain 15·5 per cent. of nitrogen and traces of iron and sulphur, but no phosphorus. It gives no Molisch reaction, and, according to Berman, no biuret action. Collip and Clark's product, however, gives the common protein reaction, the Pauli reaction, and is destroyed by pepsin and trypsin, so that it bears a strong resemblance to insulin. According to Davies, Dickens and Dodds, the active principle also forms an insoluble picrate and hydrochloride when treated by Dudley's method for the purification of insulin.

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